A STUDY OF MANGANESE RETENTIONS IN CHILDREN

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Recent studies by Skinner, Peterson and Steenbock ('31), Orent and McCollum ('31), and Kemmerer, Elvehjem and Hart ('31) have confirmed the earlier hypotheses of McHargue ('26) and McCarrison ('27) to the effect that manganese is an essential constituent of the animal organism. Rations containing less than the minimum amounts result in impotency in rats (Orent and McCollum), congenital debility in the young,[1] and possibly a failure in mammary gland development in the parturient female (Orent and McCollum). The addition of manganese to rations lacking in this element resulted in a marked increase in retention (Skinner, Peterson and Steenbock), especially during fetal life; indeed, the storage of manganese during fetal development as well as in the postnatal period seems to be of signal importance to the developing organism. But, apart from the fact that diets lacking in manganese result in testicular atrophy, the specific role it plays in the well-being of the organism as well as the relative amounts needed in the various phases of the life cycle are unknown. In rats the margin of safety in food appears to lie between 1.7 and 2.3 mg. per 100 calories.[1]

Since the manganese content of the more usual foods used by man differs so widely, ranging from the small amounts in milk (average 0.03 mg. per liter) and protein foods in general, to much higher levels in certain vegetables and whole

[1] Unpublished data.

grain cereals, it is possible that some diets consisting largely of milk and refined cereals, used more largely for children, may contain too little. It therefore seemed desirable to determine the amount of manganese retained by children at various levels of ingestion.

EXPERIMENTAL PROCEDURE

The study was made with seven children, three girls and four boys, ranging in age from 3 to 5 years. Each observation included a preliminary period of at least 7 days, and with one exception, a metabolism period of 10 days. From the beginning, the children received a constant weighed diet. The procedure in the care of the children, preparation of food and collection of stools and urine were the same as that reported in a previous study from this laboratory (Daniels and Wright, '34).

Three diets containing different amounts of manganese were selected for study, the choice of food being based on the published analyses of Hodges and Peterson ('31), Peterson and Skinner ('31), and Remington and Shiver ('30). The low and intermediate manganese diets (diets A and B) differed only in the choice of cereal foods, white bread and (with one exception) oatmeal being used in the former, and with the latter commercial whole wheat bread and a whole wheat breakfast cereal. In the high manganese diets (diet C) besides the whole grain cereal foods, high manganese-containing fruits and vegetables, blueberries, pineapple and beets were substituted for those containing the lesser amounts in the other two diets, namely, carrots, apples and prunes. All three diets contained milk, meat, eggs, bananas, potatoes, tomatoes, orange juice and cod liver oil.

The manganese in the food and stools was determined by the method of Davidson and Capen ('29). One day's aliquot of the food for each child, and the stools for the entire metabolism period were dried to constant weight on the water-bath and subsequently in a Freas oven, ground in a Pyrex mortar, and ashed in an electric furnace, such amounts of

the dried material being used as would give satisfactory readings when matched with standards containing from 0.2 to 0.25 mg. of manganese. Triplicate determinations were made in all cases, a known amount of manganese being added to the third sample to test the technic by recovery. In the few instances where recoveries fell below 93 per cent, the determinations were repeated. Distilled water and all reagents used in the analyses were found by test to contain no manganese.

Children's urines are so low in manganese that it was found necessary to make the tests with the pooled 10-day excretions. After thorough mixing, the total amount was divided in halves and these duplicates were concentrated as far as possible on the steam bath, heated repeatedly with concentrated nitric acid until the organic material was driven off, and subsequently treated with repeated amounts of concentrated sulphuric acid to remove the chlorides. The residues were then dissolved in water, the subnatant fluid decanted, and the process repeated a second time. The total solutions were concentrated to 100 cc., 0.3 gm. of potassium periodate was added to each, and enough solid sodium hydroxide to bring the mixtures to more nearly the neutral point. These were then brought to the boiling point and matched with standard permanganate solutions. Duplicate determinations were thus made with all urines.

RESULTS

The manganese content of the various diets taken by the children shows considerable variation—from 0.101 mg. per kilogram of body weight at the low ingestion level, to 0.304 mg. per kilogram at the high level (table 1). Of this the larger proportion was excreted by way of the gut. The percentage of ingested manganese excreted in the stool (79 per cent) was the same with the two diets (diets B and C) containing the larger amounts of manganese. At the lower ingestion level (diet A) a considerably larger percentage (92 per cent) was excreted. This was somewhat surprising,

since it was anticipated that the diets containing the larger amounts of manganese might result in a lower percentage of absorption because of the amount of roughage contained therein. The alimentary excretion would seem to be in part metabolized manganese, as well as that which was unabsorbed. The manganese of the urine was exceedingly small, depending

TABLE 1

The ingestion, excretion and retention of manganese

NAME	DATE	AGE	WEIGHT	INTAKE		OUTPUT			RETENTION	
				Total	Per kilogram	Urine	Feces	Total	Total	Per kilogram
Diet A. Low in Manganese										
		months	*kg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
D.B.	1/10	64	18.62	1.885	0.101	0.006	1.690	1.696	0.189	0.010
L.B.	1/10	61	15.54	1.888	0.121	0.007	1.761	1.768	0.120	0.008
V.L.	1/10	60	16.19	1.890	0.117	0.007	1.696	1.703	0.187	0.012
B.S.	10/24	50	15.00	1.690	0.113	0.008	1.518	1.526	0.164	0.011
Average		59	16.36	1.836	0.113	0.007	1.666	1.673	0.165	0.010
Diet B. Intermediary in Manganese										
B.S.	1/30	53	14.35	2.151	0.150	0.007	1.792	1.799	0.352	0.024
L.B.	2/1	62	16.19	2.265	0.140	0.009	2.036	2.045	0.220	0.014
P.H.	1/30	44	16.56	2.221	0.134	0.007	1.890	1.897	0.334	0.020
F.G.	10/24	52	14.52	2.502	0.172	0.010	1.967	1.977	0.525	0.036
J.P.	10/24	46	17.63	3.435	0.195	0.013	2.350	2.363	1.072	0.061
Average		51	15.85	2.515	0.158	0.009	2.007	2.016	0.501	0.031
Diet C. High in Manganese										
D.B.	12/11	63	17.97	5.226	0.291	0.013	4.077	4.090	1.136	0.063
L.B.	12/11	60	15.71	4.976	0.317	0.012	4.103	4.115	0.861	0.055
V.L.	12/11	59	15.54	4.873	0.314	0.012	3.936	3.948	0.925	0.060
Average		61	16.40	5.025	0.307	0.012	4.038	4.051	0.974	0.059

in part on the intake, the lowest amount (0.006 mg.) being co-existent with the lowest intake and the larger amounts (0.012 mg.) correlating with the higher intakes.

The amount of manganese retained in the studies herein reported was found to be higher at the higher ingestion levels; for example, at an ingestion of 0.11 mg. per kilogram

the average retention was 0.010 per kilogram, whereas 0.059 mg. per kilogram was retained with an average ingestion of 0.307 mg. That the amount retained is fairly proportional to the amount ingested, within the limits of the study, is further indicated by the retentions of four children: L.B., who was studied at the three levels; and V.L., B.S. and D.B., who were studied during two ingestion levels. In all cases, more manganese was retained with the higher ingestions. For example, L.B. retained 0.008 mg., 0.014 mg. and 0.055 mg. per kilogram at ingestions of 0.121, 0.140 and 0.317 mg. per kilogram, respectively, while V.L. retained 0.012 mg. and 0.060 mg. per kilogram with ingestions of 0.117 and 0.314 mg. per kilogram. J.P., on the other hand, retained as much manganese at an ingestion level of 0.195 as other children at somewhat higher levels. His diet at the intermediary manganese level consisted of a larger proportion of whole wheat cereal than that of the other children of diet B group, thus making for a larger ingestion of manganese and a correspondingly higher retention.

The variations in the manganese retentions of the children studied at the several levels may be due in part to their previous store and in part to the difference in their individual physiologic needs. But the fact that consistently higher retentions were obtained at the higher ingestion levels in children who had received fair amounts previously would seem to indicate that there is a definite manganese need which may not be met by diets which appear adequate from other standpoints. In the age group studied this would seem to lie between 0.20 and 0.30 mg. per kilogram.

SUMMARY

Twelve manganese balance studies with seven children have been made at three different levels of ingestion. Within the limits of the study the amount retained was found to be proportional to the amount ingested, thus indicating that manganese is essential to the physiological development of children. It is suggested that the diet for children should contain between 0.20 and 0.30 mg. of manganese per kilogram.

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UTILIZATION OF MEAT BY HUMAN SUBJECTS [1]

I. THE UTILIZATION OF THE NITROGEN AND PHOSPHORUS OF LOIN AND HEEL CUTS OF BEEF

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For those of limited means it is customary to urge the consumption of the less tender and consequently less expensive beef cuts. Since a question has arisen concerning their relative merits as sources of nitrogen in the diet (Mitchell, '27), a balance study was made to determine the efficiency of the nitrogen from loin and heel of beef, representing a most and a least tender cut. The utilization of the phosphorus from the two meats was also studied and their collagen and elastin content determined. The experiment was divided into two parts, each preceded by a 4-day preliminary period and including two 4-day experimental periods. In the first part, the beef loin was used and in the second part, the 'heel.'

The meat was of known origin and good grade. Equal weights of loin and heel were taken from the same carcass so any observed differences in usage could be attributed to cut rather than to grade. The meats were freed from visible fat, coarsely ground once, and each thoroughly mixed. The daily supply for each subject was weighed in two portions, frozen quickly, and kept frozen until used.

The subjects were three healthy young women of 70 kg. each, using from 34.5 to 41.0 Calories per kilogram. Their diet (table 1) was base-forming. Breakfast consisted of a nitrogen-free bread, filtered butter fat and a fruit drink.

[1] Contribution no. 32, Department of Home Economics.

These same foods were eaten with the meat at lunch and dinner and two subjects also ate a fondant-like candy. Distilled water was used ad libitum. Ferric citrate and calcium carbonate were added to bring the calcium and iron up to accepted standards (Sherman, '32).

The diet furnished from 87.5 to 87.8 per cent of the daily protein requirement of 44.4 gm. per 70 kg. (Sherman, '32),

TABLE 1

Daily food intake in loin of beef period[1]

SUBJECT	FOOD	WEIGHT	PROTEIN	PHOSPHORUS	CALORIES
		gm.	gm.	gm.	
A	Meat	165.0	36.6	0.341	190
	Bread[2]				972
	Butterfat, filtered	27.0			243
	Beverage[3]	838.2	2.3	0.093	1099
	Candy[4]				365
	$CaCO_3$	1.375			
	$FeC_6H_5O_7$	0.041			
	NaCl	Ad libitum			
	Total		38.9	0.434	2869
B	Same as A, omitting candy and 10 gm. butter fat; 2414 calories.				
C	Same as A, omitting 10 gm. butter fat; 2779 calories.				

[1] In the heel of beef period the protein totaled 38.5 gm. and the phosphorus 0.432 gm. per day.

[2] Bread.	gm.
Arrowroot starch	120
Lactose	40
Dextri-maltose	15
Baking powder, tartrate	3
Sodium chloride	3
Agar-agar	4
Butter fat, filtered	32
Water, distilled	80

[3] Beverage.	gm.
Grape juice	600.0
Lemon juice	128.2
Lactose	35.0
Sucrose	75.0

[4] Candy.	gm.
Sucrose	75
Arrowroot starch	5
Butter fat, filtered	5
Water, distilled	60

the meat supplying 94.2 to 94.5 per cent of the total nitrogen and 78.2 to 78.9 per cent of the total phosphorus. The latter provided only 49.1 to 49.3 per cent of the daily phosphorus requirement of 0.88 gm. per 70 kg. of body weight (Sherman, '32).

The customary precautions were taken for care of samples. Urine was collected quantitatively for each 24-hour day and feces for each 4-day period. Total nitrogen was determined

by the Kjeldahl-Gunning method, phosphorus by the volumetric procedure of the Association of Official Agricultural Chemists, and collagen and elastin by revised directions from the Illinois Agricultural Experiment Station.

TABLE 2

Average daily nitrogen and phosphorus balances

SUBJECT	CUT OF MEAT	AVERAGE DAILY INTAKE		AVERAGE DAILY OUTPUT			BALANCE
		Meat	Total	Urine	Feces	Total	
				Nitrogen [1]			
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
A (70 kg.)	Loin [2]	5.86	6.22	4.24	1.31	5.55	+ 0.67 ± 0.10
	Heel [3]	5.82	6.16	4.95	1.41	6.36	− 0.20 ± 0.13
B (70 kg.)	Loin	5.86	6.22	5.16	0.70	5.86	+ 0.36 ± 0.12
	Heel	5.82	6.16	5.19	0.83	6.02	+ 0.14 ± 0.11
C (70 kg.)	Loin	5.86	6.22	4.00	1.47	5.47	+ 0.75 ± 0.20
	Heel	5.82	6.16	4.26	1.68	5.94	+ 0.22 ± 0.21
All subjects	Loin	5.86	6.22	4.47	1.16	5.63	+ 0.59 ± 0.08
	Heel	5.82	6.16	4.80	1.31	6.11	+ 0.05 ± 0.09
				Phosphorus [1]			
A (70 kg.)	Loin	0.341	0.434	0.320	0.944	1.264	− 0.830 ± 0.154
	Heel	0.338	0.432	0.402	1.140	1.542	− 1.110 ± 0.190
B (70 kg.)	Loin	0.341	0.434	0.349	0.712	1.061	− 0.627 ± 0.115
	Heel	0.338	0.432	0.352	0.857	1.209	− 0.777 ± 0.566
C (70 kg.)	Loin	0.341	0.434	0.279	1.155	1.434	− 1.000 ± 0.294
	Heel	0.338	0.432	0.243	1.492	1.735	− 1.303 ± 0.305
All subjects	Loin	0.341	0.434	0.316	0.937	1.253	− 0.819 ± 0.072
	Heel	0.338	0.432	0.332	1.163	1.495	− 1.063 ± 0.104

[1] Average of 8 experimental days.

[2] Collagen furnished 6 per cent of total nitrogen; elastin, 1.1 per cent.

[3] Collagen furnished 12.2 per cent of total nitrogen; elastin, 0.9 per cent.

With a daily intake of 0.089 gm. nitrogen per kilogram, the average daily retention for all subjects was + 0.59 ± 0.08 gm. for the entire 8 days of the beef loin period (table 2). This indicated good usage of loin nitrogen. Two subjects were in negative balance on each of 2 days, but only once for each was this appreciable in amount. With heel of beef supplying

0.088 gm. of nitrogen per kilogram daily, all subjects were close to nitrogen equilibrium, one being negative and the other two slightly positive. The average daily retention of nitrogen for all subjects on this cut was $+0.05 \pm 0.09$ gm., although on half of the experimental days the balances were negative.

Digestibility varied more with the subjects than with the meats. The average coefficient of digestibility for the nitrogen of the loin of beef for the various subjects ranged from 69.5 to 91.3 per cent and averaged 81.3 per cent for all subjects. In the heel period it ranged from 63.8 to 87.7 per cent with an average of 78.8. Urinary nitrogen amounted to 0.064 gm. per kilogram per day in the loin period and 0.069 gm. for the heel period, again suggesting slightly better usage of the nitrogen from the tender cut.

Collagen nitrogen, calculated as percentage of total nitrogen, was twice as high in the heel as in the loin (table 2) while the elastin nitrogen was nearly the same.. The difference in the average for the nitrogen balances for the two cuts which is 4.5 times its P. E. ($\frac{0.54}{0.12} = 4.5$) may be regarded as significant and possibly may be explained by the difference in the original collagen content of the meats and the degree to which it was converted into gelatin in cooking. It cannot be attributed to elastin, since the slight difference in this case was in favor of the less tender meat. Variations in individual subjects are also apparent in these results.

A negative phosphorus balance was obtained in all cases, as was expected with a daily intake of approximately 6 mg. per kilogram of body weight or half the actual requirement. The individual phosphorus balances for the beef loin period ranged from -0.457 to -1.365 gm. per day and averaged -0.819 ± 0.072 gm. for all subjects. The results were less favorable for the heel of beef with practically the same intake. The daily average phosphorus balance for this period was -1.063 ± 0.104 gm. and varied from -0.693 to -1.751 gm. for the different subjects. Fecal phosphorus for the heel of beef periods was slightly higher than for the loin paralleling a somewhat lower coefficient of digestibility for nitrogen and

suggesting greater phosphorus losses in digestion. Phosphorus balances were more negative for all subjects in the second period for each cut of meat indicating inability to adjust to so low an intake. The difference in the average phosphorus balances for all subjects for the two meats of -0.244 ± 0.126 gm. is not significant and suggests less difference in quality of phosphorus than was noted for nitrogen.

CONCLUSION

When heel of beef is substituted for loin in the diet as a source of nitrogen and phosphorus, the amount should be increased slightly, since these elements appear to be somewhat better used from the more tender cut. The differences for nitrogen appear to be greater than for phosphorus.

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THE SPECIFIC DYNAMIC EFFECTS OF PROTEIN, FAT, AND CARBOHYDRATE AS DETERMINED WITH THE ALBINO RAT AT DIFFERENT PLANES OF NUTRITION[1]

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INTRODUCTION

In a previous publication (Forbes, Kriss and Miller, '34) confirmatory evidence was presented to the effect that the relationship between the plane of nutrition and the heat production of the albino rat, from the fasting level to the level of one and a half times maintenance, is represented not by a straight line, but by a curve of increasing steepness of upward slope. This phenomenon may be interpreted to signify either, 1) that the specific dynamic effects of nutrients increase with the rise in plane of nutrition or, 2) that the current method of computation of the specific dynamic effects of nutrients, which relates the nutrients ingested to the resultant increase in metabolism over the basal level, needs revision.

The first suggestion of the reasonableness of the latter point of view came from Rubner as long ago as 1902, when he made the observation that the administration of phlorhizin to a fasting dog caused an increase in the heat production coincident with an increase in body protein katabolism, as compared with the normal fasting values.

[1] Publication authorized by the director of the Pennsylvania Agricultural Experiment Station, March 16, 1934, as technical paper no. 644.

Rubner considered the results of this experiment as a demonstration of the existence of a 'specific dynamic effect' of body protein, when katabolized, similar to that of food protein. This view seems to have been accepted, in part at least, by Lusk, for in reviewing this work ('28) he stated that "this increase (in heat production) Rubner rightly attributed to the specific dynamic action of increased (body) protein metabolism."

In accord with this conception Williams, Riche and Lusk ('12) proposed a method of calculating the specific dynamic effect of protein which accounted for that of the body protein katabolized during fasting by relating the extra protein metabolized, instead of the total protein ingested, to the increase in the heat production over the basal level. This principle, however, has been disregarded by many workers in the field.

In a critical analysis of published data, largely those of Lusk and his pupils, Borsook and Winegarden ('31 a) observed that the specific dynamic effect of protein parallels the course of nitrogen excretion. These authors have shown that when the increase in heat production following the ingestion of proteins or amino acids is compared with the increase, over the basal level of excretion, of urinary nitrogen, a close correlation is obtained, while failure to account for the basal nitrogen excretion, or for the incompleteness of metabolism of the nitrogen ingested, might be the cause of anomalous results. These observations of Borsook and Winegarden, which find support in the work of Wilhelmj and Bollman ('28), Wilhelmj and Mann ('30), Wilhelmj, Bollman and Mann ('31), Wishart ('28) and of Wilhelmj ('34) recall the early idea of Rubner ('02) that when body tissue is katabolized it exerts a specific dynamic effect, which is, of course, not the same as its heat of oxidation.

Rubner's contention that body protein and ingested protein have the same specific dynamic effect and that the higher basal metabolism in the phlorhizinized dog is the result of the specific dynamic effect of the increased destruction of body

protein finds support in the observations by Chambers and Lusk ('30) that the heat production rises to the same height after the administration of meat to the normal dog and afterward to the same dog when phlorhizinized. Additional evidence in favor of this explanation is found in the experiments by Dann, Chambers and Lusk ('31) with thyroidectomized dogs.

In seeking an explanation for the curvilinear relationship between the heat production and the plane of nutrition, Forbes, Braman and Kriss ('28, '30) and later, Forbes and Kriss ('32) concluded—in harmony with the ideas expressed above—that the fasting heat production is greater than the hypothetical minimum energy requirement by the amount of the waste heat of the body nutrients katabolized during fast; that the heat production between the fasting and the maintenance levels is affected by this factor in progressively diminishing degrees; and that the heat production at or above the maintenance level is entirely unaffected by this factor.

The purpose of the present investigation was to throw further light on these conclusions, and to explain the differences observed in experiments with cattle, between the dynamic effects of food above and below maintenance.

EXPERIMENTAL PROCEDURE

The subjects employed in this investigation were albino rats weighing about 100 gm. at the beginning of the experiments. Several series of respiration experiments were conducted as follows:

1. For the determination of the specific dynamic effects of protein, carbohydrate and fat, at supermaintenance planes nine female rats were used, all of which were subjected to the following dietary treatments in the different experimental periods (table 1): 1. Maintenance basal ration consisting of 5.5 gm. per day of a commercial mixed calf meal and estimated to maintain the animals in approximate energy equilibrium. 2. Basal ration (as in 1) plus 2 gm. per day of casein (Harris). 3. Basal ration (as in 1) plus 2.2 gm. per day of

cornstarch. 4. Basal ration (as in 1) plus 1 gm. of olive oil per day. The quantities of casein, starch and oil added to the basal ration were approximately equicaloric, and together with the basal ration constituted an excess of energy over the maintenance requirement equivalent to approximately one-half of the latter.

The calf meal used as the basal ration was of the same composition as given in the preceding paper.

TABLE 1

Schedule of supermaintenance respiration experiments, and body weights

RAT NO.	BASAL RATION 5.5 GM.		BASAL RATION PLUS 2 GM. CASEIN		BASAL RATION PLUS 2.2 GM. STARCH		BASAL RATION PLUS 1 GM. OLIVE OIL	
	Date	Body weight	Date	Body weight	Date	Body weight	Date	Body weight
	1931	*Gm.*	1931	*Gm.*	1931	*Gm.*	1931	*Gm.*
1199	Nov. 16	108	Nov. 23	123	Nov. 30	126	Dec. 7	132
1301	Nov. 18	106	Nov. 25	121	Dec. 2	127	Dec. 9	132
1300	Nov. 20	98	Dec. 3	119	Dec. 10	124	Dec. 17	126
	1932		1932		1932		1932	
387	Jan. 4	104	Jan. 14	124	Jan. 20	128	Jan. 28	138
386	Jan. 6	99	Jan. 11	113	Jan. 18	122	Jan. 25	132
388	Jan. 7	98	Jan. 13	111	Jan. 21	126	Jan. 27	132
385	Jan. 8	91	Jan. 15	106	Jan. 22	114	Jan. 29	
451	Oct. 3	98	Oct. 11	104	Oct. 19	115	Oct. 24	124
453	Oct. 3	99	Oct. 11	106	Oct. 18	117	Oct. 24	125

2. For the determination of the specific dynamic effects of protein, carbohydrate and fat at the maintenance level six female and eight male rats were used. All of these animals were subjected to a basal metabolism test. Five of the female rats and five of the male rats were subjected, in different periods, to an exclusive protein diet consisting of 3.8 gm. per day of casein (Harris); four of the female rats and five of the male rats were subjected to an exclusive carbohydrate diet consisting of 3.92 gm. of cornstarch daily; and three of the female and four of the male rats were subjected to an exclusive fat diet consisting of 1.56 gm. daily of olive oil. The quantities of casein, starch and oil fed in this series

were estimated to maintain the animals in approximate energy equilibrium. As will be seen from the schedule of experimentation (table 2), the majority of the animals were subjected, at different times, to two experimental dietary treatments in addition to fasting. In only one case were all four experimental treatments imposed on the same animal (rat no. 34). Although no obvious ill effects on the animal were observed in this case, the number of experimental treatments imposed on the same animal was limited to three in all other

TABLE 2

Schedule of maintenance respiration experiments, and body weights

RAT NO.	SEX	CASEIN (3.8 GM. PER DAY)		STARCH (3.92 GM. PER DAY)		OLIVE OIL (1.56 GM. PER DAY)		FASTING	
		Date	Body weight	Date	Body weight	Date	Body weight	Date	Body weight
		1932	*Gm.*	1932	*Gm.*	1932	*Gm.*	1932	*Gm.*
61	F	Mar. 14	111	Mar. 21	108	Mar. 25	84	Mar. 28	106
62	F	Mar. 16	104	Mar. 23	102			Mar. 30	103
64	F	Mar. 18	82			Mar. 25	84	Apr. 1	90
63	F					Mar. 24	90	Mar. 31	97
58	M			Apr. 13	94	Apr. 19	87		
57	M			Apr. 14	92	Apr. 20	88	Apr. 25	97
59	M			Apr. 15	91			Apr. 26	97
56	M			Apr. 18	94			Apr. 21	97
34	F	Aug. 8	96	Aug. 15	95	Aug. 22	87	Aug. 29	93
38	M	Aug. 17	101	Aug. 26	94			Sept. 1	98
35	M	Aug. 18	97	Aug. 25	94			Sept. 7	92
36	F	Aug. 19	111	Aug. 30	108			Sept. 7	114
31	M	Sept. 2	98			Sept. 9	90	Sept. 13	94
37	M	Sept. 6	103			Sept. 14	92	Sept. 19	100
33	M	Sept. 8	100			Sept. 15	93	Sept. 20	100

cases in order to avoid any possible ill effects of prolonged confinement to the restricted and incomplete diets employed.

All of the diets used were analyzed for energy, and the basal ration and casein were also analyzed for nitrogen. In special metabolism tests with control animals the urinary nitrogen excretion was determined for each diet; and the metabolizability of the energy of the basal diet (calf meal) and of the casein was determined in special balance experiments.

The rats were fed individually, twice daily, and were kept on the experimental diet for at least 3 days prior to the respiration experiments. During the intermediate or transition periods the rats received a maintenance ration of the basal diet. The respiration measurements began as soon as the morning feed was consumed, and, in the case of fasting, they began 24 hours after the last portion of feed was given. The respiration experiments were usually conducted for 7 hours, during which the hourly CO_2 production and the total respiratory quotients were determined, by means of the apparatus and general technic described in the preceding paper (Forbes, Kriss and Miller, '34).

The heat production was computed on the basis of, 1) the hourly CO_2 production (omitting the weights of CO_2 for the first 2 hours, for reasons explained in the preceding paper); 2) the protein metabolism, as measured by the urinary nitrogen excretion of control animals and, 3) the non-protein respiratory quotients. As in the previous investigation all the data for heat production were computed to a basis of 100 gm. of empty body weight, the empty weights of the experimental animals being calculated on the basis of the data for alimentary fill of rats, under similar dietary treatments reported elsewhere by Miller and Kriss ('34).

DISCUSSION OF RESULTS

Heat production and respiratory quotients

The hourly heat production of the rats, under the different dietary conditions, and the corresponding respiratory quotients and statistical constants, are presented in tables 3 to 5, inclusive. Table 3 contains the data for the rats which received the basal maintenance ration alone, and for those which received this ration supplemented with casein, starch or olive oil. Tables 4 and 5 contain the corresponding data for the male and female rats, respectively, while fasting, and while receiving the maintenance diets of casein, starch, or olive oil alone. The data for the hourly heat production of the individual rats are all presented in consecutive order.

TABLE 3

Hourly heat production of female rats, per 100 gm. of empty body weight, and respiratory quotients as influenced by the addition of casein, starch, and olive oil to a basal maintenance ration [1]

RAT NO.	BASAL RATION (5.5 GM. CALF MEAL)			BASAL RATION PLUS 2 GM. CASEIN			BASAL RATION PLUS 2.2 GM. STARCH			BASAL RATION PLUS 1 GM. OLIVE OIL		
	Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.	
		Total	Non-protein		Total	Non-protein		Total	Non-protein		Total	Non-protein
1199	649 651 651 607 689	0.93	0.96	754 678 678 709 903	0.97	1.18	791 771 771 807	0.97	1.01	710 754 754 738 822	0.91	0.93
1301	654 639 639 654 677	0.96	1.00	761 761 827 823 778	0.97	1.16	737 737 618 836 617	0.98	1.02	695 704 704 848 881	0.92	0.95
1300	672 687 687 756 761	0.94	0.97	779 732 732 679 691	0.93	1.10	695 708 720	1.05	1.11	684 700 700 782 715	0.88	0.90
387	642 645 645 585 721	0.95	0.99	766 877 877 772 771	0.95[2]	1.12	679 679 651 696	1.03	1.09	635 800 800 719 718	0.89	0.91
386	635 662 662 702 727	0.95	0.99	786 787 787 801 781	0.97	1.17	783 713 713 779	1.06	1.12	740 762 762 706 741	0.91	0.93
388	654 672 672 620 677	0.96	1.00	780 770 780	0.95	1.13	886 886 882 877	1.01	1.05	795 792 792 828 833	0.87	0.88
385	603 604 603 612	0.96	1.00	779 779 817 786	0.96	1.15	833 705 705	1.00	1.04	581 651 652 763 699	0.88	0.90
451	669 669 622 638	0.95	0.99	771 748 748 771 695	0.93	1.10	743 743 712 658	1.05	1.11	686 712 712 622	0.92	0.95
453	741 741 684 639	0.91	0.94	784 770 770 737 705	0.95	1.15	681 562 660	1.08	1.15	655 641 641 624	0.93	0.96
Mean	662± 4.4	0.95	0.98	769± 5.1	0.95	1.14	739± 8.7	1.02	1.08	727± 6.9	0.90	0.92
Standard deviation of means	6.5± 0.49			7.6± 0.56			12.9± 1.07			10.2± 0.75		
Standard deviation	42.1± 3.18			49.2± 3.63			75.2± 6.24			66.9± 4.92		

[1] The quantities of casein, starch and olive oil were not exactly equicaloric. The influence on the average daily heat production of these nutrients on an equicaloric basis is shown in table 5.

[2] Assumed.

Attention is called to the fact that the rats were unusually quiet in the chamber after it was placed in the water bath under the electric light. The animals were, however, unusually restless while they were being weighed in the closed jar, thus causing an excessive accumulation of CO_2. For this reason, principally, the CO_2 measurement for the first hour of the experimental period was always excessively high, and with the rate of ventilation (about 35 liters per hour) maintained in the present series of experiments a partial carrying over of this effect into the second hour was also evident in most cases. On this account, the CO_2 measurements during the first 2 hours of the experimental periods were omitted from the computations of the heat production.

This procedure is especially justified by the fact that our data do not show any rise in heat production during the first few hours after feeding such as is usually observed during realimentation following a period of fasting. In other words, under our conditions of experimentation the heat production of the animals, unaffected by irregularities in activity, is not curvilinear, but is practically on a level. In a subsequent experiment in which the ventilation was at a higher rate than that maintained in the present series we have actually observed that within less than an hour after feeding (a supermaintenance diet) the heat production was brought to a level which was practically maintained throughout the experimental period of 12 hours.

Feeding the animals twice a day did not allow them to be reduced to a fasting state during the intervals between feeding, and no very large fluctuations in the respiratory quotients during the experimental periods could, under the conditions, be expected. Hence, any errors in the computation of the individual hourly heat production by the use of the factors for the average respiratory quotient obtained during the experimental period should be relatively small.

Because the experimental periods were fairly long, the average respiratory quotients possess a high degree of accuracy. This is evidenced by the uniformity in the respiratory quotients for the individual animals on the same diets.

The mean hourly heat production (table 4) for the fasting female rats (518 calories) was almost identical with the corresponding mean (table 5) for the male rats (520 calories). In the previous study (Forbes, Kriss and Miller, '34) the average hourly heat production for a group of eight male

TABLE 4

Hourly heat production of female rats, per 100 gm. of empty body weight, and respiratory quotients during fast and when receiving equicaloric quantities of casein, starch or olive oil exclusively

RAT NO.	FASTING			CASEIN (3.8 G.M. PER DAY)			STARCH (3.92 GM. PER DAY)			OLIVE OIL (1.56 GM. PER DAY)		
	Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.	
		Total	Non-protein		Total	Non-protein		Total	Non-protein		Total	Non-protein
61	499 610 611 577 522	0.69	0.65	736 672 672	0.82		601 560 560 504 561	1.11	1.13			
62	454 500 500 457 506	0.74	0.72	681 685 769 755	0.82		620 620 559 591 504	1.07	1.09			
64	470 516 516 601 656	0.75	0.73	719 742 742 729 778	0.83					546 561 561 568 606	0.70	0.68
63	502 455 455 484 515	0.74	0.72							510 533 533 551 604	0.72	0.71
34	544 546 546 574	0.73	0.71	639 581 581 584 621	0.82[1]		617 589 588 660 612	1.05	1.07	621 540 505 505 557	0.71	0.70
36	602 421 445 445 496	0.73	0.70	653 659 648 648 649	0.82		594 539 552 552 546	0.99	1.00			
Mean	518± 7.4	0.73	0.71	679± 8.5	0.82		576± 5.9	1.06	1.07	553± 6.0	0.71	0.70
Standard deviation of means	10.9± 0.98			12.6± 1.31			8.7± 0.95			8.9± 1.13		
Standard deviation	58.7± 5.28			59.1± 6.14			38.9± 4.25			34.5± 4.38		

[1] Assumed.

TABLE 5

Hourly heat production of male rats, per 100 gm. of empty body weight, and respiratory quotients during fast, and when receiving equicaloric quantities of casein, starch or olive oil exclusively

RAT NO.	FASTING			CASEIN (3.8 G.M. PER DAY)			STARCH (3.92 GM. PER DAY)			OLIVE OIL (1.56 GM. PER DAY)		
	Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.		Cals.	R.Q.	
		Total	Non-protein		Total	Non-protein		Total	Non-protein		Total	Non-protein
57	519 509 509 553 551	0.75	0.73				643 664 664 615 596	1.10	1.12	562 623 623 599 599	0.73	0.72
59	564 514 514 520 591	0.75	0.73				606 615 615 590 605	1.11	1.13			
56	507 531 532 500 549	0.76	0.75				614 639 639 640 638	1.07	1.09			
38	524 549 538 538 482	0.72	0.69	763 723 723 741 713	0.82		545 568 584 584 622	1.11	1.13			
35	591 564 562 562 615	0.75	0.73	702 702 634 672	0.84		572 613 635 635 597	1.07	1.09			
33	470 472 456 456 491	0.72	0.69	645 625 640 640 632	0.81					492 473 479 480 490	0.69	0.67
31	555 463 463 451	0.74	0.72	708 662 628 629 640	0.78					599 501 591 591 598	0.70	0.68
37	517 485 507 506 491	0.74	0.72	594 619 648 648 622	0.83					530 505 543 543 502	0.70	0.68
Mean	520± 4.3	0.74	0.72	665± 6.1	0.82		614± 3.8	1.09	1.11	546± 7.8	0.71	0.69
Standard deviation of means	6.4± 0.50			9.1± 0.90			5.7± 0.55			11.6± 1.27		
Standard deviation	40.0± 3.12			44.6± 4.41			28.5± 2.75			51.9± 5.68		

rats was found to be 530 calories. While the differences are in favor of the male rats, they are too small to be considered significant, and the agreement between the data representing the two different groups of male rats may be taken as an indication that the conditions of experimentation were satisfactorily controlled.

Both the total and the non-protein respiratory quotients are given in the tables. The computation of the non-protein respiratory quotients, and of the protein metabolism, in all experimental periods except those in which casein was fed, was based on Loewy's ('11) factors, which are generally used. These factors are:

1 mg. of urinary nitrogen is equivalent to 4.75 cc. of CO_2
1 mg. of urinary nitrogen is equivalent to 5.94 cc. O_2
1 mg. of urinary nitrogen is equivalent to 26.51 calories

The computation of the protein metabolism in the casein experiments was based on factors experimentally determined by Kriss and Miller ('34) for casein. These are:

1 mg. of urinary nitrogen is equivalent to 5.47 cc. CO_2
1 mg. of urinary nitrogen is equivalent to 6.67 cc. O_2
1 mg. of urinary nitrogen is equivalent to 30.59 calories
1 liter of respiratory O_2 is equivalent to 4586 calories

While the protein metabolism was taken into consideration in all of the calculations of the heat production in these experiments, a study of the experimental data revealed that the added accuracy gained by so doing was of minor degree. A comparison of the average hourly heat production as computed from the total and from the non-protein respiratory quotients gave differences ranging from 0 to 3.7 per cent, and, with the exception of the starch experiments, the figures computed from the non-protein respiratory quotient were the lower.

The non-protein respiratory quotients in the fasting experiments averaged 0.71 (table 4) and 0.72 (table 5). These data indicate that 24 hours after the last feeding the rats had reached a status of essentially true fast. The non-protein respiratory quotients in the olive oil periods were slightly

lower than those obtained during fast, suggesting that the oil feeding probably resulted in slight ketosis.

Non-protein respiratory quotients somewhat higher than unity were obtained in the periods in which starch or casein was added to the basal ration (table 3), as well as in the period in which starch was fed exclusively (tables 4 and 5). It appears that 3.92 gm. of starch per day was slightly more than sufficient to supply the energy need for the maintenance of the rats under the experimental conditions, and that the addition of 2 gm. of casein or of 2.2 gm. of starch to the basal ration of calf meal rendered some carbohydrate available for conversion into fat.

In the periods in which casein was fed exclusively (tables 4 and 5) the total respiratory quotients average 0.82, with only slight variation. These respiratory quotients, representing mainly protein metabolism, are identical with the respiratory quotient found by Kriss and Miller ('34) to represent the metabolism of pure casein. The average non-protein respiratory quotient may therefore be taken to be essentially the same as the total respiratory quotient. Since the proportions of non-protein material metabolized in these periods were relatively very small, the non-protein respiratory quotients for the individual rats were considered, in these cases, to be without special significance, and were therefore omitted from the tables.

Percentage contributions of heat from protein, fat and carbohydrate and the sparing of body nutrients by food nutrients

Preliminary to calculations of the specific dynamic effects of the various nutrients studied, calculations were made (table 6) of the proportions of the total heat production derived from protein katabolism, carbohydrate katabolism, fat katabolism, and from the synthesis of fat from carbohydrates, under the different dietary conditions. The derivation of these data was based on the urinary nitrogen excretion of control animals, the respiratory quotients, and the total heat produc-

tion. These data are of especial significance in showing the sparing effect of one nutrient upon the other, and have an important bearing on the determination of the specific dynamic effects of these nutrients.

Thus, it will be observed that while during fast the protein metabolism contributed 26.0 per cent to the heat production, this percentage dropped to 5.6 in the case of starch feeding.

TABLE 6

Urinary nitrogen excretion and percentage contributions of heat from protein, fat and carbohydrates to the total heat production

DAILY FOOD INTAKE	NITROGEN METABOLISM TESTS			AVERAGE PERCENTAGE OF TOTAL HEAT PRODUCED BY			
	Number of animals	Urine collection periods, days	Average milligrams urinary nitrogen per day [1]	Protein katabolism	Carbohydrate katabolism	Fat katabolism	Fat synthesis
Basal ration (5.5 gm. calf meal)	7	2	112 ± 3.9	18.7	76.1	5.2	....
Basal ration plus 2 gm. casein	6	2	333 ± 5.7	55.2	43.5	....	1.3[2]
Basal ration plus 2.2 gm. starch	4	2	108 ± 4.2	16.1	82.6	....	1.3[2]
Basal ration plus 1 gm. olive oil	4	2	102± 3.6	15.5	62.6	21.9	....
Fasting	10	1	122 ± 4.2	26.0	....	74.0	....
3.8 gm. casein	5	3	508 ± 6.2	96.4	1.5	2.1	....
3.92 gm. starch	4	2	30 ± 1.2	5.6	92.6	....	1.8[2]
1.56 gm. olive oil	4	2	70 ± 2.5	14.1	....	85.9	....

[1] Per 100 gm. empty body weight.

[2] When the non-protein respiratory quotient is above 1.00, for each 5.047 calories non-protein katabolized, 1.09× (respiratory quotient —1) calories are synthesized (Lusk, G., Science of Nutrition, 4th ed., p. 397).

In absolute amounts the daily protein metabolism was reduced from 3234 calories during fast to 795 calories when 3.92 gm. of starch were fed; or, in terms of urinary nitrogen, the reduction was from 122 mg. during fast to 30 mg. in the starch feeding periods. In the later case, therefore, the protein metabolism dropped to about one-fourth of the fasting value. Similarly, when 1.56 gm. of olive oil was fed to the

rats, the protein metabolized (70 mg. urinary nitrogen) was only slightly more than one-half of the fasting protein metabolism, the contribution to the total heat production being 14.1 per cent.

If there is a dynamic effect of body nutrients katabolized, therefore, the apparent specific dynamic effects of carbohydrate or of fat, as determined under any conditions such that they spare the katabolism of body nutrients, must be values of mixed significance.

There is evidence in the literature that the elaboration and excretion of urinary nitrogen is attended by a considerable expenditure of energy. This subject has been adequately discussed by Borsook and Winegarden ('31 a, b, c) and by Borsook and Keighley ('33 a, b). If this is true, the ideal condition for determining the specific dynamic effect of a non-protein nutrient, involving the comparison of the heat production in two experimental periods, would be that the protein metabolism be the same in the two periods compared. As will be observed, such a condition practically prevails when the comparison is made between a maintenance ration and the same ration plus a non-protein supplement, but does not prevail in any comparison involving the fasting metabolism.

Furthermore, it is not to be expected that the so-called sparing effect of non-protein food nutrients on the body protein metabolism would be the same regardless of the quantity of the substance fed. On the contrary, this effect may be expected to vary greatly with the quantity of such food nutrients.

The foregoing considerations imply that more consistent and presumably more significant values for the specific dynamic effects of carbohydrates and fats might be obtained if the differences in protein metabolism between the periods compared could be taken into account, or if the heat production of the animal maintained in energy and protein equilibrium, instead of the fasting heat production, be made the base value for comparison with higher levels.

The feeding of casein naturally resulted in large increases in protein metabolism. When 3.8 gm. of casein was fed practically all of the absorbed nitrogen (540 mg.) was metabolized and excreted in the urine, contributing 96.4 per cent of the total heat production. In this case all of the urinary nitrogen (508 mg.) is to be considered as representing casein metabolism.

The feeding of casein, therefore, in quantity sufficient to supply the entire maintenance requirement of energy and of protein, completely protected the body protein from katabolism. This sparing effect of protein feeding, or the replacement of body protein by food protein, should be taken into consideration in determinations of the specific dynamic effects of proteins.

Thus, when a comparison is made, as is usual in determinations of the specific dynamic effects, between the heat production of fasting and the heat production of the protein feeding, this comparison, in relation to the amount of casein under discussion, is as between zero casein intake and 3.8 gm. casein intake, but in relation to protein metabolism, it is not as between zero protein metabolism and the metabolism of 3.8 gm. casein, because during fast some protein (from the body) is metabolized, which involves work by the kidneys, and an expenditure of energy aside from the heat of oxidation of protein per se.

Above the maintenance requirement, as when comparing the basal ration (5.5 gm. calf meal) with the same ration plus any of the supplements, there is, of course, no replacement of body nutrients by food nutrients to consider, since the basal ration alone was adequate to protect the animal from loss of body tissue.

The addition of starch or olive oil to the basal maintenance ration had a relatively slight effect on the amount of protein metabolized. The addition of casein to the basal maintenance ration resulted in an increase in urinary nitrogen (110 mg. per gram of casein), which, in relation to the casein added, is similar in magnitude to the increase observed (102 mg. per

gram of casein) between fasting and 3.8 gm. of casein, but markedly different from the value (134 mg. per gram of casein) which expresses the relationship between the 3.8 gm. of casein and the corresponding total urinary nitrogen excretion.

In the foregoing discussion of the effects of feeding carbohydrate, fat and protein at different planes of nutrition, particularly in the discussion of their sparing effect on body protein, as affecting the determination of the specific dynamic effects, it was assumed that body tissue, when metabolized, gives rise to a specific dynamic effect. A test of this hypothesis is found in the comparison of the specific dynamic effects of casein, starch and olive oil as determined by the different methods discussed below.

Specific dynamic effects of casein, starch and olive oil at supermaintenance and at maintenance planes of nutrition

For the purpose of determining the specific dynamic effects of the casein, starch and the olive oil tested, the average hourly heat production of the rats under the various dietary treatments, given in tables 3, 4 and 5, was first of all computed to a 24-hour basis. This computation is justified by the observation that the heat production is practically on a level, due, in large measure, to the facts that the rats were fed twice daily and that they were established on the respective experimental dietary regimes for several days prior to the respiration measurements. In other words, the several hours' heat measurements are considered, under the experimental conditions, to be fairly representative of the daily heat production.

The specific dynamic effects of casein, starch and olive oil, at supermaintenance planes of feeding (table 7) were computed from a comparison of the average daily heat production of the rats on 5.5 gm. of the basal maintenance ration with their average daily heat production on the same ration plus the respective supplements. In this computation, therefore, the heat production of maintenance, and not of fast, was taken as the base value.

The ration of 5.5 gm. of calf meal was found, in energy and nitrogen balance experiments, to be required for the maintenance of the body weight of a 100-gm. rat under cage conditions, while under the conditions of more restricted activity in the respiration chamber, 5.5 gm. of calf meal was found to supply some excess of energy over the quantity required to maintain energy equilibrium.

The metabolizable energy value of the casein fed in these experiments was also determined from complete balances of energy and nitrogen (Kriss and Miller, '34), while the computation of the metabolizable energy of the starch and of the

TABLE 7

Specific dynamic effects of casein, starch and olive oil when added to a basal maintenance ration

BASAL RATION	DAILY SUPPLEMENT	METABOLIZABLE ENERGY OF SUPPLEMENT	AVERAGE DAILY HEAT PRODUCTION PER 100 GM. OF EMPTY BODY WEIGHT	INCREASE IN HEAT PRODUCTION CAUSED BY SUPPLEMENT	
				Total	Per 100 Cals. of metabolizable energy ingested
		Cals.	*Cals.*	*Cals.*	*Cals.*
5.5 gm.			15,888		
5.5 gm.	2 gm. casein	8166	18,456	2568	31.4
5.5 gm.	2.2 gm. starch	8228	17,736	1848	22.5
5.5 gm.	1 gm. olive oil	9426	17,448	1560	16.5

olive oil was based on the assumption that these substances are completely digested. This assumption is justified mainly by the chemical nature of these substances, sustained by the fact that when either was fed exclusively, to the control groups used in the nitrogen metabolism experiments, no feces, or negligible quantities of feces, were dropped during the observational periods.

The increase in heat production caused by the addition of 2 gm. of casein to the basal maintenance ration (table 7) was 2568 calories. This heat increment is 31.4 per cent of the metabolizable energy of the ingested casein.

The addition of 2.2 gm. of starch (8228 calories) to the basal maintenance ration caused an increase in the heat production of 1848 calories, or 22.5 per cent of the metabolizable energy of the starch.

The addition of 1 gm. of olive oil (9426 calories) to the basal maintenance ration resulted in a heat increment of 1560 calories, or 16.5 per cent of the metabolizable energy of the oil.

In the light of the foregoing discussion, these increases in heat production may be considered the true specific dynamic effects of these substances. Casein exhibits the highest effect, olive oil shows the lowest, while starch takes an intermediate place. This order of magnitude has been shown generally to

TABLE 8

Specific dynamic effects of casein, starch and olive oil when each of these was fed exclusively to female and male albino rats

DAILY FOOD	METABOLIZABLE ENERGY OF DAILY FOOD	AVERAGE DAILY HEAT PRODUCTION PER 100 GM. OF EMPTY BODY WEIGHT		INCREASE IN HEAT PRODUCTION OVER FASTING							
				Total		Per 100 calories of metabolizable energy					
						Uncorrected		Corrected for sparing effect on body protein		Corrected for sparing effect on body protein and fat	
		Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
	Cals.	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*	*Cals.*
Fasting	.....	12,432	12,480	....	....	....	....	....	....	....	....
3.8 gm. casein	15,515	16,296	15,960	3864	3480	24.9	22.4	31.5	28.3	36.5	32.2
3.92 gm. starch	14,661	13,824	14,736	1392	2256	9.5	15.4	14.7	20.1	20.2	24.4
1.56 gm. olive oil	14,705	13,272	13,104	840	624	5.7	4.2	8.7	6.9	14.1	11.2

prevail in comparisons of the specific dynamic effects of proteins, fats and carbohydrates, though the actual values for carbohydrate and fat differ considerably from those given by Lusk ('28).

Turning to a consideration of the determination of the specific dynamic effects of casein, starch and oiive oil at maintenance levels of feeding (table 8), using the fasting heat production as the base value, for comparison—it will be observed that the metabolizable energy of the daily amounts of casein, starch or olive oil ingested exceeded the daily fasting

heat production, but approximated fairly closely the quantities of heat produced in the respective feeding periods. In other words, the quantities fed represented approximately the maintenance requirements of energy. It was, of course, impossible without a previous knowledge of the specific dynamic effects of the substances tested, to determine the exact quantities required to provide energy equilibrium.

The specific dynamic effects of the casein, the starch and the olive oil were computed by two procedures, first, the method usually followed by students of this subject, by relating the increase in heat production over the fasting metabolism to the metabolizable energy ingested; and, second, by relating to the metabolizable energy the same heat increment corrected for the sparing of body tissue, which has been mentioned. The results obtained with the male and the female rats are given separately.

The fundamental reasons for the necessity of accounting for the sparing of the body protein by foods, in determination of these heat increments, have been previously discussed, and evidence was cited which justifies the assumption that the specific dynamic effect of body protein broken down is of the same order of magnitude as that of food proteins. On the basis of these considerations and of the data of table 6, the corrections for the sparing of body protein by casein, starch and olive oil, respectively, were made as follows:

In respect to fasting, the 3.8 gm. of casein spared 122 mg. of body nitrogen per day, which represent 3234 calories of metabolizable energy. This quota is subtracted from the metabolizable energy of the casein, in order to determine the increase in metabolizable energy of protein which would be referable to the increase in heat production over the fasting metabolism. The specific dynamic effect of the casein is in this case, therefore, corrected for the sparing of body protein indirectly by relating the observed increase in heat production to the metabolizable energy of the protein ingested diminished by the metabolizable energy of the protein broken down during fast.

The 3.92 gm. of starch and the 1.56 gm. of olive oil spared, in relation to fasting, 92 mg. and 52 mg. of body nitrogen, respectively, which represent 2439 calories and 1379 calories of metabolizable energy, respectively. The specific dynamic equivalents of the protein spared are computed on the basis of the specific dynamic values of casein (corrected as above for the sparing of body protein), and are added to the respective increases in heat production over the fasting metabolism. These corrected heat increments are then related to the metabolizable energy of the nutrients ingested.

All values for the specific dynamic effects are expressed as calories per 100 calories of metabolizable energy.

The specific dynamic values computed for the male and female rats show fair agreement, the values for casein and olive oil being somewhat higher with the female rats than with the male rats, while the reverse is true in the case of starch. Apparently these differences in specific dynamic effect are not closely, if at all, related to the sex. The average values for the two groups are, therefore, considered more significant than the values representing either one of these groups.

The heat increment values of either casein, starch, or olive oil determined with respect to fasting (table 8), and uncorrected for the sparing of body protein, are considerably lower than the corresponding values determined above maintenance (table 7).

All heat increment values determined with respect to fasting, with correction for the sparing of body protein, are considerably higher than the corresponding uncorrected values, and in the case of casein the corrected values (averaging 29.9 per cent) are only slightly lower than the specific dynamic effect (31.4 per cent) of this nutrient determined above maintenance.

The average of the heat increment values of starch, corrected for the sparing of body protein (17.4 per cent), while considerably higher than the average of the uncorrected values (12.5 per cent), is still appreciably lower than the

specific dynamic value of starch (22.5 per cent) as determined above maintenance, although the corrected value for the male rats (20.1 per cent) does not differ greatly from the specific dynamic value of starch as determined above maintenance.

The heat increment value of olive oil as determined below maintenance, with correction for the sparing of body protein (averaging 7.8 per cent), is also found to be considerably lower than the corresponding value of olive oil (16.5 per cent), as determined above maintenance.

In the last two columns of table 8 are given values for the specific dynamic effects of casein, starch and olive oil which have been corrected both for the sparing of body protein and for the sparing of body fat. The latter correction represents an attempt to account for any differences between the amounts of body fat katabolized during fast and during feeding of starch, casein, or olive oil, and rests entirely on hypothetical grounds.

In the fasting periods the metabolism of body fat constituted 74.0 per cent of the total heat production (table 6). In the periods in which starch or olive oil was fed, no body fat was broken down. In the casein periods only 2.1 per cent of the total heat production was derived from body fat. On the assumption that the metabolism of body fat produces a specific dynamic effect like that of food fat, the correction for the sparing of body fat by the various nutrients ingested was made by adding the specific dynamic equivalents of the calories of fat spared (calculated on the basis of the corrected specific dynamic values of olive oil) to the respective increases in heat production over fasting, corrected for the sparing of body protein, and relating the heat increments thus corrected to the metabolizable energy of the corresponding nutrients ingested.

It is of much interest to find that the specific dynamic values of casein, starch and olive oil, corrected for the sparing of body protein and fat, agree reasonably well with the corresponding values of these substances determined (as in table 7) above maintenance.

The writers wish to make it clear that in making the foregoing calculations of the different corrections for the specific dynamic effect of body tissue their primary aim has been to subject the various assumptions made, which in their opinion are reasonable, to experimental verification.

In the opinion of the writers, the results obtained contribute to the confirmation of the conclusion of Forbes, Braman and Kriss ('30), from experiments with cattle, that the fasting heat production includes a factor of waste heat of utilization of body tissue broken down, and to the justification of their conclusion that heat increments of rations determined below maintenance are less than the true energy expense of food utilization by the amount of the waste heat of utilization of body nutrients katabolized.

The present understanding of the writers, therefore, is that specific dynamic effects of nutrients should be measured in accord with the principle of the current method of determination, by Forbes and associates, of heat increments of rations for body increase, with the heat production of a status of nitrogen and energy equilibrium as the base value.

This procedure, the general plan of which has been designated and discussed as the feed-and-plane-difference method, was used in its earliest form, by Armsby, many years ago, in experiments with cattle, but without the present understanding of principles involved, and without a definite base value.

In the further development of this method of study of energy metabolism, the difference in heat increments above and below maintenance was established by Forbes, Fries, Braman and Kriss ('26).

The establishment and explanation of the curve of heat production, by Forbes, Braman and Kriss ('28 and '30) and by Forbes and Kriss ('32), in experiments with cattle, yielded the present understanding of the cause and significance of this difference in heat increments, as relating to entire foodstuffs or rations; while the present paper extends this understanding to its relation to proteins, fats and carbohydrates, as individual food constituents, and also definitely specifies that

the basal maintenance ration must provide for nitrogen as well as energy equilibrium. The principle of this last requirement has been understood, as expressed by Forbes ('29, '32, '33), and has been satisfied in the use of this procedure, but has not been emphasized heretofore in the present connection.

In the preceding paper (Forbes, Kriss and Miller, '34) attention was called to the different attitudes expressed by different investigators toward the curve of heat production in relation to the plane of nutrition; and to the different procedures used for the determination of the energy cost of food utilization at different planes of nutrition.

In this connection reference was made to the facts that Mitchell and Hamilton ('32), Wiegner and Ghoneim ('31), and Brody and Procter ('33) calculated the heat increments and net energy values of rations at different planes of nutrition, including supermaintenance planes, by using the fasting heat production as the base value, and in so doing employed the data of Forbes, Braman and Kriss ('28, '30) in ways implying change of interpretation from that of the authors, with the result that they obtained values of mixed significance.

It is the belief of the writers that the clearing up of this existing confusion of thought in the field of energy metabolism depends upon—and is impossible without—an effective recognition of the fact first brought to light by Rubner and substantiated by the last experimental work of Lusk and by the results of the present study that when body tissue is katabolized it is not employed for energy production at 100 per cent efficiency, but gives rise to a waste heat of utilization, and is therefore characterized by a dynamic effect, in the same sense as is food nutriment.

SUMMARY AND CONCLUSIONS

The average specific dynamic effects resulting from the addition, to a basal maintenance ration, of casein, starch and olive oil, respectively, expressed as percentages of the metabolizable energy of these supplements, were 31.4 per cent for

casein, 22.5 per cent for starch and 16.5 per cent for olive oil. These values were considerably greater than the corresponding increases in heat production, over the fasting metabolism, resulting from the exclusive feeding of casein, starch and olive oil, when these heat increments were related to the metabolizable energy ingested.

As a contribution to the establishment of the causes of these differences in heat increment values determinations were made, at the different planes of nutrition, of the percentages of the total heat derived from protein, fat and carbohydrate, and of the sparing of body nutrients, under the different dietary treatments.

Attempts were made to confirm the existence of specific dynamic effects of body protein and fat katabolized by accounting for the sparing effects of starch, olive oil and casein, on the katabolism of body tissue.

All heat increment values determined with respect to fasting, with correction for the sparing of body protein, were found to be considerably higher than the corresponding uncorrected values.

The heat increment values of casein, starch and olive oil, corrected for the sparing of both body protein and fat, were found to agree reasonably well with the heat increment values of these substances determined above maintenance—with the heat production of energy and nitrogen equilibrium as a base value.

These conditions (of determination above maintenance) are believed to be as nearly correct as practicable for measuring the specific dynamic effect of any nutrient.

The results obtained constitute fairly satisfactory confirmation of the conclusion of Rubner that the heat produced by the katabolism of body protein includes a factor of waste heat of utilization; and also justify the conclusion of Forbes, Braman and Kriss that heat increment values of rations determined directly with reference to the fasting heat production (uncorrected for the sparing of body tissue) are lower than the true energy expense of utilization by the amounts of the dynamic effect of body substance spared.

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THE ENERGY METABOLISM OF THE ALBINO RAT IN RELATION TO THE PLANE OF NUTRITION[1]

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THREE FIGURES

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INTRODUCTION

In recent investigations on the energy metabolism of cattle, Forbes, Braman and Kriss ('28, '30) determined, with four steers, the relation of the heat production to the plane of nutrition.

The heat produced by the animal, as related to the food consumed, was found to increase at an accelerating rate from fasting to the maintenance level, and to be higher above than below maintenance; but the rate of increase diminished, in all cases, between the two highest points of observation.

Two attitudes toward this curve of heat production have been expressed by students of nutrition, and two interpretations are implied by procedures which have been employed in the determination of the energy values of rations.

According to one of these positions, the energy cost of utilization of the food, at any level of intake, should be computed by relating the total rise in heat production, above that of fasting, to the quantity of food eaten.

This position, however, seems to the writers to ignore the energy expense of utilization of body nutrients katabolized—first brought to light by Rubner ('02), and the facts as to the

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origin of the heat from different proportions of protein, fat and carbohydrate, as affected by the plane of nutrition, as shown by Forbes and Kriss ('32 a, b).

In accounting for the shape of the curve and for the low heat increments of the food between planes of fasting and maintenance, Forbes, Braman and Kriss ('28, '30), and Forbes and Kriss ('32 a) concluded that the heat production of fasting includes two factors, 1) a net or dynamic factor—a hypothetical minimum value including no waste heat of utilization, and, 2) a waste or thermic factor, representing energy expense of utilization.

This second factor they understand to diminish with rise in the plane of nutrition, from its undermined value at fasting to zero at the point of energy equilibrium.

They recognized that heat increments[2] of food used above maintenance, for body increase, may properly be considered to measure the energy expense of utilization of this part of the total ration; and concluded that heat increments between the lower planes of nutrition are less than the true energy expense of food utilization, by the amount of the waste heat of utilization of body nutrients katabolized.

They further proposed that the most satisfactory method of recognizing the facts as to the significance of the curve of heat production, in the determination of energy values of food, is to compute separate heat increments for maintenance and for body increase—the former based on the heat production of fasting, and the latter on the heat production at the point of energy equilibrium.

Wiegner and Ghoneim ('31) have reported an experiment, with a rabbit, on the utilization of the energy of a mixed diet at five different planes of nutrition, including, among others, fast and one supermaintenance plane. These authors found, by using the fasting katabolism as a measure of the maintenance requirement, and as a base value for the determination of the net energy of the ration at the different levels of

[2] In this paper the term heat increment is used synonymously with the term specific dynamic effect.

intake, a progressive decrease in the percentage utilization of the metabolizable energy of the ration from the lowest to the highest level.

These observations on the rabbit are in substantial agreement with the observations made by Forbes and associates on steers, if the latter are computed in the same way. Wiegner and Ghoneim, however, made no distinction between base value for maintenance and base value for body increase, and they express the opinion that the efficiency of utilization of the ration is determined by the need of the animal for the nutriment.

Møllgaard ('31), in a critical discussion of this subject, took the position that the energy cost of food utilization for body increase is a constant, and that the utilization of the food energy below maintenance is uninfluenced by the amount of food consumed. In defense of his position, he assumed that the results of the experiments of Wiegner and Ghoneim, and of Forbes and associates, which have been cited, especially their fasting katabolism data, are in error, and averred that the reported heat increments based on the fasting data constitute a mathematical illusion. Møllgaard, however, reports no determinations of his own of the heat production of fasting animals.

Mitchell and Hamilton ('32) recently reported observations on the heat production of a single steer at six levels of nutrition, ranging from fast to full feed. The relation between the heat production and the dry matter of food consumed is represented by a zigzag line. This line shows a very slight rise in heat production from fasting to the lowest level of feeding, which was approximately half-maintenance. From this point to higher levels the heat production increased rapidly, but irregularly. Mitchell and Hamilton assign no significance to the irregularities in the increase of heat production above the second level of feeding (approximate maintenance), and characterize the rise from this point to full feed as approximately linear.

If it were true that the heat production above maintenance is a linear function of the amount of food, it would seem illogical to object to the computation of heat increments for body increase with the heat production of maintenance as the base value, but Mitchell and associates do take this position, and have computed the heat increments at the higher levels of feeding by using the heat production of fast as a base value.

Brody and Procter ('33) also compute heat increments, as measures of the energy expense of food utilization, for production as well as for maintenance, with the fasting heat production as the base value—each heat increment for a different level of production, therefore, having a different significance, because each would represent a different proportion of food utilized (at different energy expense for maintenance and for body increase).

In the light of these observations, it appeared desirable to determine the curve of heat production in relation to the quantity of the food, with the albino rat, for comparison with the equivalent curve for cattle, thus to gain further evidence as to the significance of the relationship so expressed.

EXPERIMENTAL PROCEDURE

Respiration experiments were conducted with eight male albino rats on a stock diet at three levels of food intake, namely, 8 gm. per day, 6 gm. per day, and 4 gm. per day, and also in the postabsorptive fasting condition. Eight gm. of this diet constituted full-feed, while approximately 5 gm. was required to maintain energy equilibrium. The animals were placed on experiment when they weighed about 100 gm., and full-feed was the first dietary treatment.

The stock diet consisted of a commercial calf-meal which was employed as a conveniently available and approximately complete diet. Its gross composition was: 300 parts linseed oil meal, 400 parts corn meal, 200 parts ground malted barley, 440 parts wheat red dog flour, 240 parts dried skim milk, 300 parts oat flour, 60 parts soluble blood meal, 20 parts salt, 20

parts ground limestone and 20 parts steamed bone meal. The calf-meal was sifted through a 20-mesh sieve, to remove coarse particles. The remaining portion, which alone was used, contained 3.51 per cent N, 2.92 per cent ether extract, 6.24 per cent ash, 10.48 per cent moisture, and 58.42 per cent carbohydrate (by difference). It contained 4047 calories of gross energy and 3145 calories of metabolizable energy per gram. The estimated respiratory quotient of this product was 0.94.

The rats were placed in individual cages of galvanized screen, with false bottoms to prevent coprophagy. The daily amounts of food were given in two equal portions—one at 8 A.M. and the other at 4.30 P.M. The animals were established on the desired plane of food intake prior to the respiration experiments by feeding them at the desired rate for not less than 3 days.

The method of feeding outlined was adopted in this study in the light of the writers' experience in similar studies with cattle, and on account of preliminary experimentation with rats, which showed that when the animals are kept on a restricted diet, and are fed twice during 24 hours, for a number of days, their metabolism becomes fairly constant, and the heat production of a part of the day, under the existing conditions of restricted activity, represents the whole reasonably well.

The respiration experiments were usually of 7 hours' duration. In the experiments in which the animals received feed the rat was placed in the respiration chamber as soon as the morning portion was consumed, which was usually within an hour after the time of feeding. In a few cases when the rats were slow in cleaning up the food, the experimental periods were necessarily shortened.

For the determination of the fasting katabolism, the rat was first established on a plane of approximate energy equilibrium, and the respiration experiment was started 24 hours after the last portion of feed was given.

The sequence of the dietary treatments was as follows: Full feed, one-half feed, three-quarters feed, and fasting. The dates of the respiration experiments, and the initial weights of the animals are presented in table 1.

The apparatus used was devised after the Haldane principle, the moisture and carbon dioxide production, and the oxygen consumption, being determined gravimetrically. Separate measurements on two rats could be made simultaneously. The details of this apparatus are shown in figures 1 and 2.

TABLE 1

Schedule of respiration experiments, and body weights

RAT NO.	8 GM. BASAL RATION		4 GM. BASAL RATION		6 GM. BASAL RATION		FASTING	
	Date	Body weight	Date	Body weight	Date	Body weight	Date	Body weight
	1932	*Gm.*	1932	*Gm.*	1932	*Gm.*	1932	*Gm.*
457	Nov. 11	111	Nov. 17	101	Nov. 28	116	Dec. 2	107
458	Nov. 11	115	Nov. 16	107	Nov. 28	120	Dec. 2	110
459	Nov. 21	139	Nov. 25	122	Nov. 29	126	Dec. 5	119
460	Nov. 14	123	Nov. 23	111	Nov. 29	121	Dec. 5	110
461	Nov. 15	127	Nov. 23	108	Nov. 30	120	Dec. 6	111
462	Nov. 21	124	Nov. 25	111	Nov. 30	117	Dec. 6	109
463	Nov. 17	117	Nov. 22	103	Dec. 1	114	Dec. 7	106
464	Nov. 16	128	Nov. 22	110	Dec. 1	124	Dec. 8	115

As a means of control of activity, in the interest of comparable results, the rat was placed in a cylinder of ¼ inch mesh galvanized screen, of a fit sufficiently close to prevent the animal from moving about with freedom. The rat in the wire cylinder was then placed in a 1-quart Mason jar; the lid was screwed on loosely, and the jar, rat and wire cylinder were weighed together on a large analytical balance. Weighing to an accuracy of 1 mg. was usually accomplished within 5 minutes. Immediately after weighing, the lid of the jar was replaced by a rubber stopper, provided with connections of rubber tubing for attachment to the ingoing and outcoming air lines of the respiration apparatus. The stoppered jar was placed horizontally in a weighted wooden frame; was im-

mersed in a constant-temperature water bath, maintained at 28.2°C.; and then the connections were made between the chamber and the air circuit.

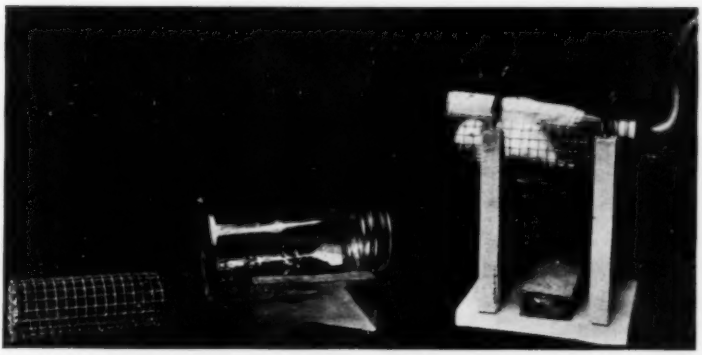

Fig. 1 Mason jar used as a respiration chamber for rats, and a galvanized screen cylinder used to control the activity of the animal.

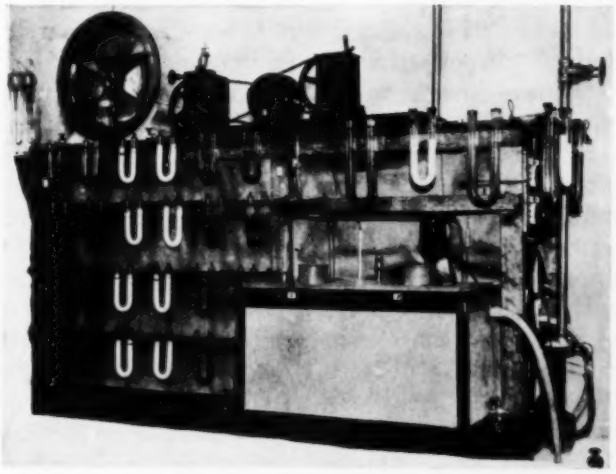

Fig. 2 Respiration chamber and accessory equipment, for indirect calorimetric experimentation with rats.

The water bath was covered by a lid in the center of which was a 25-watt electric light so placed as to illuminate the chamber below it. The purpose of the light was to incline the rat to keep its eyes shut, and therefore to remain quiet.

No observations were made of the temperature of the air in the chamber, but it is safe to assume that it was only slightly higher than that of the surrounding water, and that it was above the critical point during all metabolism measurements, 28°C. having been found by Benedict and MacLeod ('29) to be the critical temperature for the fasting rat. Outside of the respiration apparatus the rats were kept in a room at a temperature of about 27°C., which was exceeded only in very warm weather.

The rate of ventilation was maintained, during this series of experiments, at 35 to 40 liters per hour, by means of a double-stage rotary pump, designed primarily for vacuum work; and the air supply was measured by a Bohr meter. The rate of ventilation was maintained constant during experiments, in order to minimize possible differences in composition of the air in the chamber at the end as compared with the beginning of experimental periods.

The carbon dioxide was collected in U-tubes containing soda lime, and moisture was collected in U-tubes containing sulphuric acid and finely divided pumice stone. Two sets of such absorption tubes were connected with the outcoming air line from the chamber, to be used alternately, 1 hour at a time, except that at noon one set of tubes was used for 2 hours, as a matter of convenience. The length of experiments, with some exceptions, was 7 hours.

The oxygen consumption during experimental periods was determined as the difference between the loss in weight of the animal in the chamber and the gain in weight of the absorption tubes, due to the moisture and carbon dioxide produced by the animal. Respiratory quotients were determined from the total carbon dioxide produced and the total oxygen consumed, the error in using total instead of non-protein respiratory quotients having been found practically negligible.

The heat production was computed from the average respiratory quotient for the experimental period and the hourly carbon dioxide production. The weight of carbon dioxide of the first hour, and sometimes that of the second hour also, was omitted from the computation of the heat (but not from the computation of the R. Q.'s) because of the accumulation of carbon dioxide which occurred in the chamber while the animal was being weighed. The errors in the heat production due to the use of the average respiratory quotients are considered to be relatively small, in view of the fact that under the experimental conditions no very large variations in the respiratory quotients could be expected, especially, in consideration of the intervals between feeding and of the observed rates of CO_2 production.

DISCUSSION OF RESULTS

Table 2 gives the hourly heat production in consecutive order and the total respiratory quotients for the individual rats in the different experimental periods. For the purposes of comparison and statistical treatment, all of the data for heat production are expressed as calories per 100 gm. of empty weight, this basis of reference being considered more significant than the gross body weight.

The determination of the empty weight of the experimental animals, by the method of Miller and Kriss ('34), was based on parallel determinations on control animals which received the same dietary treatment. Thus it was assumed that the initial empty weights of the experimental subjects with food intake of 8 gm., 5.5 gm., and 4 gm., and with no food, were 88.74 per cent, 89.98 per cent, 90.58 per cent, and 96.93 per cent, respectively, of the gross weights. The proportion of empty to gross weight of rats which received 6 gm. was assumed to be the same as with rats which received 5.5 gm. of food.

Since the empty weight of each of the animals was about 100 gm. (table 1), the computation of the heat production to the basis of 100 gm. empty weight involved but small cor-

TABLE 2

Hourly heat production of male rats, per 100 gm. of empty body weight, and total respiratory quotients, as influenced by the plane of nutrition

RAT NO.	FASTING		4 GM. BASAL RATION PER DAY		6 GM. BASAL RATION PER DAY		8 GM. BASAL RATION PER DAY	
	Cals.	*R.Q.*	*Cals.*	*R.Q.*	*Cals.*	*R.Q.*	*Cals.*	*R.Q.*
457	596 601 547 547 575 653	0.75	727 632 632 681 604	0.89	825 777 653 653 697 711	0.96	848 851 701	0.90
458	544 497 546 547 653 539	0.73	688 584 643 643 691 625	0.88	653 666 701 701 668 673	0.98	912 912 788 738 858	0.93
459	538 569 587 587 535	0.75	701 657 603 603 668 668	0.90	787 699 652 652 647 777	0.99	892 885 885 805 749	1.00
460	464 488 486 486 534	0.74	618 625 547 547 577 609	0.94	688 702 703 643 736	0.99	804 774	1.01
461	605 621 490 490	0.74	632 718 606 606 619 636	0.92	705 645 611 611 649 652	0.98	905 936 810	0.93
462	524 500 477 477 525	0.72	564 538 538 625 583	0.89	704 600 606 606 679	0.98	757 790 721 721 718 785	1.01
463	478 534 461 461 447 450	0.74	548 548 535 558	0.93	661 626 626 656	1.01	755 772 823	0.96
464	550 475 511 511 575 520	0.75	615 589 589 586 594	0.91	626 644 645 604	0.95	980 980 996 785 781	0.91
Mean	530± 5.4	0.74	614± 5.1	0.91	672± 5.31	0.98	826± 9.6	0.96
Standard deviation of means	8.0± 0.59		7.6± 0.56		7.9± 0.59		14.3± 1.22	
Standard deviation	52.5± 3.87		49.8± 3.67		51.2± 3.83		80.9± 6.90	

rections, and was considered satisfactory for the purposes in view.

The surface area law, and the exponential relationship between body weight and metabolism, were recognized by computing the corrections for the differences between the actual empty weight and the standard weight of 100 gm., not in direct proportion, but in proportion of the two-thirds power of these weights.

The data of table 2 reflect the effectiveness of the experimental technic. The heat measurements for consecutive hours were fairly uniform, and a satisfactory degree of uniformity was also found among the respiratory quotients. Of the factors which have contributed to the uniformity of these measurements, the following deserve especial emphasis:

1. Restriction of the activity of the rat while in the respiration chamber. All who have attempted to use rats for respiration experiments recognize that the activity of the subjects constitutes a serious problem. Some workers have attempted to overcome this difficulty by making records of the activity of the animals, to serve as a basis of selection among the experimental data, but for obvious reasons this is not a satisfactory solution. On the other hand, the limitation of space for the rat in the chamber, and the bright illumination, in the experiments here discussed, were found highly satisfactory as a means of placing the metabolism measurements on a comparable basis. It is true that when first inclosed in the cylinder, a rat is restless; but it soon accommodates itself, and then remains remarkably quiet.

2. Establishing the animals on the desired plane of food intake prior to the respiration experiments. The determination of dynamic effects, or heat increments, from established planes of feeding is equivalent to the determination of the distance between two parallel lines (representing the heat production on two planes of nutrition), which may be accomplished readily and with certainty. In other words, the heat production is not curvilinear, but is practically on a level. This procedure, which has been employed in all determina-

tions of heat increments with cattle at this institute, has marked practical advantages over the determination of the heat increment due to a single feeding.

3. Feeding twice a day. The advantage of dividing the daily ration into two equal portions, and feeding one in the morning and one in the evening, is twofold: The food is eaten promptly, thus making it possible to conduct the heat measurement at a convenient, predetermined time; and, what

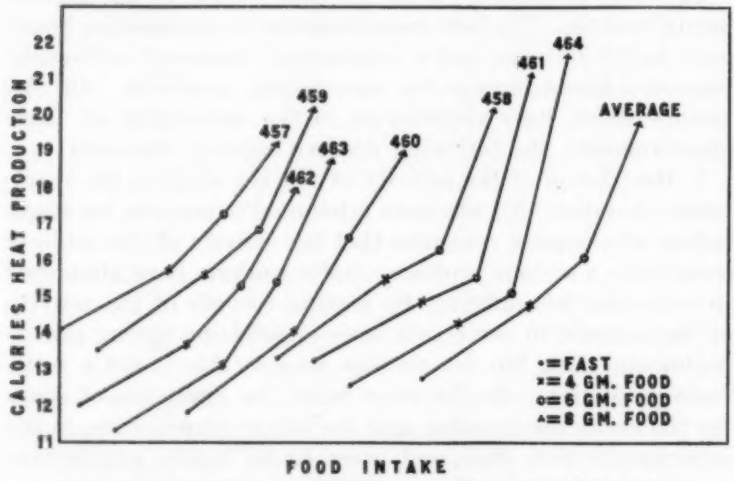

Fig. 3 The heat production of albino rats related to the food intake.

is more important, this method of feeding makes for uniformity of metabolism throughout the day.

In figure 3 are plotted the calories of heat production of the individual rats, computed to a 24-hour basis (table 3) against the grams of food intake per day. The average results from the eight animals, similarly plotted, are represented in this figure by a separate curve which is considered as the most significant. Each of these curves shows a greater rate of increase in metabolism from the 6-gm. to the 8-gm. level than from the fasting to the 4-gm., or the 6-gm., or the 8-gm. level.

With one exception (rat no. 460), the increase from the 6-gm. to the 8-gm. level was greater than from the 4-gm. to the 6-gm. level. Five gm. of food supplied the approximate energy maintenance requirement of the animals.

TABLE 3

Daily heat production of individual rats per 100 gm. of empty body weight, on different planes of nutrition

RAT NO.	FASTING	4 GM. BASAL RATION	6 GM. BASAL RATION	8 GM. BASAL RATION
	Cals.	*Cals.*	*Cals.*	*Cals.*
457	14,088	15,720	17,256	19,200
458	13,296	15,504	16,248	20,208
459	13,512	15,600	16,848	20,232
460	11,808	14,088	16,656	18,936
461	13,248	14,880	15,504	21,216
462	12,024	13,680	15,336	17,976
463	11,328	13,128	15,408	18,792
464	12,576	14,280	15,120	21,696

The average curve representing the relationship between the plane of nutrition and the heat production shows a striking similarity to the corresponding curves obtained by Forbes and associates with steers. Of particular interest is the rather marked break in the curve at about the maintenance level. The average numerical values for the heat increments, per gram of food, between various levels (table 4), bring out this point even more strikingly.

TABLE 4

Average daily heat production of rats on different planes of nutrition, and the resulting heat increments

FOOD INTAKE	HEAT PRODUCTION PER DAY PER 100 GM. OF EMPTY BODY WEIGHT	PLANES OF NUTRITION COMPARED	HEAT INCREMENTS PER GRAM OF FOOD
	Cals.		*Cals.*
Fasting	12,720	Fast and 4 gm. basal ration	504
4 gm. basal ration	14,736	Fast and 6 gm. basal ration	568
6 gm. basal ration	16,128	Fast and 8 gm. basal ration	888
8 gm. basal ration	19,824	4 gm. and 6 gm. basal ration	696
		4 gm. and 8 gm. basal ration	1272
		6 gm. and 8 gm. basal ration	1848

If the fasting heat production is used as the base value for the computation of the heat increments, these values rise progressively from 504 calories per gram of food eaten, at the 4-gm. level, to 888 calories at the 8-gm. level; but when the heat increments are computed between consecutive points of observation, the increase from the 4-gm. to the 6-gm. level is 696 calories; and from the 6-gm. to the 8-gm. level is 1848 calories per gram.

In comparing these curves for rats with the equivalent curves for steers, it should be borne in mind that at the highest planes of nutrition the steers ate from two to three times as much feed as required for maintenance, whereas the rats ate a maximum of only about one and three-fifths times as much feed as required for maintenance. It seems, therefore, that they did not eat enough, and that there were not points of observation sufficient in number, to show the slight reverse curvature that was found at the higher planes of nutrition with each of the four steers used as subjects, this reverse curvature having been interpreted as the result of diminished metabolizability of the food at the higher planes of nutrition.

It would be unilluminating and, indeed, unwarranted to assume, as did Møllgaard ('31) that errors in the determination of the fasting katabolism of steers, by Forbes and associates, are the chief cause of the relatively low heat increments, as these are computed with reference to the fasting heat production. Møllgaard's assertion that these determinations of the fasting heat production were too high, on account of food residues in the alimentary tract, was not based upon significant evidence, and finds no support in the analysis of the heat production of these animals by Forbes and Kriss ('32 a, b) which showed that in the fasting periods the non-protein material katabolized consisted entirely of fat.

Opposed to Møllgaard's assertions that the determinations of fasting heat production of steers, by Forbes and associates, are too high, and that values computed with reference to these determinations constitute only a mathematical illusion, are other experimental data of Forbes, Braman, Kriss

and Swift ('31), and analogous data of Benedict and Ritzman ('27), representing the fasting katabolism of steers, and the corresponding respiratory quotients, which show that the true fasting status is reached, with cattle, between the second and the fourth day of fast.

The values alluded to by Møllgaard as illusory are illusory only if misunderstood and misinterpreted. The experimental data of Forbes and associates, with steers as subjects, are characterized, in the writers' opinion, by a high degree of validity, and it is their belief that when the contribution to the curve of heat production of the dynamic effects of body nutrients katabolized is correctly appreciated it will be understood that heat increments for maintenance, as computed with reference to the fasting heat production do not express the true, physiological, energy expense of food utilization, but are in reality conventional values, and are so computed only because there is no other base value representing the status of no-feed-intake which is as definite as is a properly standardized determination of the heat production of fast. Forbes, Braman, Kriss and Swift ('31) have adopted terms for such a standardization.

There is an apparent inconsistency in Møllgaard's criticism ('31) of Wiegner and Ghoneim's data dealing with the effect of the plane of nutrition on the utilization of the metabolizable energy of the ration, since, on the one hand, he recognized the finding of a greater energy cost of food utilization (or a lower net energy value) for body increase than for maintenance; but, on the other hand, assuming in this case also, as in the work of Forbes and associates with steers, that the value for the fasting katabolism of the experimental animal (rabbit) was too high, he recalculated the data in such a way as to show that there is no difference in the efficiency of utilization of the energy of the ration at levels above and below the maintenance level.

That the metabolism data for the fasting rats (table 2) represent true fasting conditions is shown by the facts that the total respiratory quotients are between 0.72 and 0.75 (the

non-protein respiratory quotient would be slightly lower), and that practically no food residue was found in the stomachs and small intestines of control animals, in a status of fast corresponding to that of the subjects of the respiration experiments. The relatively low heat increments obtained in comparisons involving the fasting heat production (table 4), therefore, must be accepted as matters of fact, but they must not be interpreted as representing the true energy expense of food utilization.

The results of this study confirm, in a general way, the relations between the quantity of feed eaten, and the heat produced, as determined by Forbes and associates with cattle, and by Wiegner and Ghoneim with the rabbit. There is also some evidence, in work of Gigon ('11), that the relationship of food to heat production is quite similar in the case of man. It seems likely, therefore, that the same physiological principles prevail, in this relation, in these different species.

Experimental work bearing on the specific causes for the apparent differences in the energy cost of food utilization at different planes of nutrition will be discussed in subsequent papers.

SUMMARY AND CONCLUSIONS

Respiration experiments, by the open-train Haldane procedure, were conducted with eight male albino rats, of approximately 100 gm. empty body weight, at four planes of nutrition, ranging from fasting to full feed. The same diet—a commercial, mixed calfmeal—was used throughout. The rats were fed twice daily, and were established on the desired plane of food intake prior to the respiration measurements. The measurement of the fasting metabolism was started 24 hours after the last meal was given.

The activity of the rat was restrained, during respiration experiments, by inclosure in a rather close fitting galvanized screen cylinder within the chamber.

The hourly heat production and the total respiratory quotients varied but little for any given dietary treatment.

The average daily heat production per 100 gm. of empty body weight was 12,720 calories for fasting, 14,736 calories with 4 gm. of food; 16,128 calories with 6 gm. of food, and 19,824 calories with 8 gm. of food per day.

The average increments in heat production between fast and the 4-, 6- and 8-gm. food levels, respectively, were 504, 568 and 888 calories per gram of food consumed. Much higher heat increment values were obtained in comparisons which did not involve the fasting heat production. The highest value, 1848 calories per gram, was obtained between the two highest levels of feeding, which were above maintenance.

The results of this study confirm the observations of Forbes and associates on steers, of Wiegner and Ghoneim on the rabbit, and of Gigon on man, in showing a progressive increase in the rate of rise of the heat production with increase in food consumption, within certain limits. These results and others refute Møllgaard's allegation that the comparatively low heat increments between the fasting and the maintenance planes, found by Forbes and associates, and by Wiegner and Ghoneim, are due to experimental errors in the fasting heat measurements.

The same general relationship apparently exists between food consumption and heat production in the rat, the rabbit, the steer, and the human being.

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THE EFFICACY OF VITAMIN D ADMINISTRATION IN AQUEOUS PREPARATION

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FOUR FIGURES

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INTRODUCTION

For experiments which we were conducting in 1931 on in-vitro calcification of bone we found it necessary to make aqueous preparations of vitamin D. The following series of experiments was arranged to determine the efficacy of these preparations on growing animals rendered appropriate for the purpose by a diet deficient in calcium as well as in phosphorus. We have used the rat for all our studies whether of the living animal or of bone fragments in-vitro despite the general conviction that the rat, during its growing period, is relatively less dependent than the growing child upon vitamin D, for these studies are designed to inquire into the principle rather than the degree of dependence.

The activated ergosterol used in the experiments was generously provided by Messrs. Mead, Johnson & Co. The actual feeding and medication were carried out under our immediate supervision by Miss Dora Steinfeld who also made some of the ash determinations quoted in this essay.

The mineral-deficient diet used was the following:

	parts
Dried yellow corn	76
Wheat gluten	20
Talcum purificatum	3
Sodium chloride	1

Our determinations show a calcium content of 0.74 per cent in the ash of this diet compared with 35.3 per cent in the ash of the Steenbock and Black diet no. 2965. Therefore the lime in our special diet seems negligible. The diet will be defined in the following pages as the mineral-deficiency diet to distinguish it from the regular rachitic diets no. 2965 of Steenbock and Black and no. 3143 of McCollum, both of which were

TABLE 1

Rats weaned at 30 days were fed on the mineral-deficiency diet for the preparatory period and then utilized in the following experiments

LITTER	NUMBER OF RATS	PREPARATION PERIOD	TYPE AND PERIOD OF MEDICATION	
		days		*days*
I	9	8	Aquasterol F[1]	21
II	7	16	Aquasterol F[1]	21
III	7	8	Talc residue[2]	21
IV	7	8	Viosterol[4]	21
VI, VII	17	21	Aquasterol G[3]	32
IX, X, XI	19	35 to 39	Aqueous ergosterol[5]	33
XII, XIII, XIV	14	27	Aquasterol H[6]	25

[1] Ten cc. 10,000 X viosterol extracted with water to 75 cc.; dosage, 1 cc. daily.

[2] Ten cc. 10,000 X viosterol extracted with water to 250 cc.; dosage, 1 cc. daily.

[3] Talc residue left after extraction of F[1] suspended in 75 cc. water; dosage, 1 cc. daily.

[4] One gm. 10,000 X viosterol diluted to 25 gm. with maize oil; dosage, 5 minims (1000 r.u.) daily.

[5] One-tenth mg. crystalline activated ergosterol incorporated in 250 cc. water; dosage, 1 cc. daily.

[6] An aqueous preparation of commercial cod liver oil U.S.P.; dosage, 1 cc. daily (1 cc. aquasterol H contains the water soluble extractives of 1 cc. C.L.O.).

designed to disturb the relation between calcium and phosphorus, whereas our intention was to eliminate both minerals. On the basis of experiments by McCollum, Kruse and Orent ('33), it may be surmised that the magnesium content of the diet, in short term experiments, will prevent the elimination of mineral from the skeleton. The absence of calcium and phosphorus from the diet will greatly restrict the amount of these minerals available for the growth of the animals.

The experiments were all conducted in the following manner. White rats on weaning at 1 month of age were fed on the mineral-deficiency diet for an initial preparatory period of varying duration (table 1). They were placed singly or in pairs in metal containers covered with wire netting and black paper. The diet, finely ground, and in sufficient quantity, with fresh drinking water, was at all times available to the animals. The experimental medication was administered through a special pipette to insure adequate consumption and to control the dosage. Control litter mates were kept on the mineral-

TABLE 2

Bone ash determinations: stock diet

LITTER	DAYS AFTER BIRTH	DAYS AFTER WEANING	PER CENT ASH
XVI	13		25.8
(8 rats)	19		29.7
	27		34.3
	Weaned at 30 days		
	35	5	33.8
	42	12	38.1
	53	23	38.2
	53	23	38.6
	72	42	41.0
XVII	16		26.4
(4 rats)	23		31.9
	Weaned at 30 days		
	44	14	35.2
	75	45	43.0

deficiency diet without medication. The upper end of one tibia was split longitudinally and one-half used for histological study (Shipley et al., '20, '21). The remainder of both lower extremities (femora, tibiae and fibulae but not the feet) were dissected out, cleaned, dehydrated and defatted, then ashed to constant weight. The results are expressed as percentage of ash in dried, fat-extracted bone (Hume et al., '32).

Animals taken from litters VIII and XIV, two litters fed continuously on the deficiency diet without medication, give the ash determinations of table 3 which stand in contrast to

the figures in table 2, the ash determinations on litters XVI and XVII fed on a contrasting stock diet, made up as follows:

	parts
Whole wheat flour	66
Whole milk powder	22
Talcum purificatum	1
Sodium chloride	1
Lettuce, three times weekly	

These figures show that the several litters differ greatly in their skeletal ash percentage but nevertheless the different members of a single litter, despite segregation and allowing

TABLE 3

Bone ash determinations: deficiency diet

LITTER	DAYS AFTER WEANING	PER CENT ASH
XIV	18	34.6
(7 rats)	19	34.2
	30	31.6
	31	29.1
	31	30.2
	49	25.6
	55	26.1
VIII	30	25.8
(3 rats)	40	24.1
	45	24.8

for the individual differences in efficiency of food utilization (Palmer and Kennedy, '31), indicate the progress of skeletal mineral content.

While preparing this report for publication, we have noted the corresponding but apparently less detailed study by Beccari ('33) on the efficacy of water-soluble derivatives of vitamin D in the prevention and cure of rickets experimentally produced in white rats by McCollum diet 3143.

We have also received a copy of the report by Bourdillon and his associates ('31). This excellent study points out many pitfalls but it does not specially emphasize the fact that the rats under observation were kept upon the rachitogenic diet 2 weeks only before commencing the vitamin medication.

Our histological observations lead us to lay special stress upon this duration for we find that the bones of rats fed upon a rachitogenic diet for 19 days or more are already so pathological that the healing process can do no more than repair an area so disorganized that the structure remains permanently mutilated. Another feature to which we would draw attention is the progressive rarefaction of the shaft which can be seen on the roentgenographic scale of healing. The production of this rarefaction is discussed a little later in our report.

DETAILS OF EXPERIMENT

In the experiments recorded below are two series. The first inquires into the action of that fraction of activated ergosterol-in-oil which can be transferred to watery solution. For purposes of distinction this type of preparation is recorded as aquasterol and the letter of designation defines the concentration. The second series inquires into the action of activated ergosterol in crystalline form when utilized in watery solution.

Aquasterol F was obtained by 'extracting' 10 cc. of 10,000 X viosterol with water to a final volume of 75 cc. One cc. of this preparation was administered daily to each animal of two litters of rats (litters I and II, table 4). Litter I was fed on the mineral-deficiency diet for 8 days; litter II for 16 days. Thereafter, to both litters, medication was given along with the mineral-deficiency diet for 21 days. The animals serving as negative controls and receiving no medication show rickets by both histological and x-ray examination. The animals of litter I show no rickets after their 8-day preparation but those of litter II show early rickets at the end of 16 days before commencement of the aquasterol experiment. After 21 days of medication there is no histological evidence of rickets in any of the test animals and the degree of healing can unhesitatingly be assessed at 6 on Dyer's histological standards ('31).

The residual viosterol left from the preparation of aquasterol F was suspended in water and made up to a volume of

75 cc. One cc. of this suspension was administered daily by mouth to each animal of another litter (litter III, table 5).

TABLE 4

Bone ash determinations: deficiency diet from date of weaning; medication by aquasterol F

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
I	7	0	22.7
(9 rats)	9	1	22.2
	16	8	28.9
			31.4
			30.3
			28.4
	29	21	29.7
			36.6
			38.4 Ave. 32.5
II	14	0	26.4
(7 rats)			26.7
	27	11	35.3
			40.1
			39.6
	37	21	40.7
			40.3

TABLE 5

Bone ash determinations: deficiency diet from date of weaning; medication by talc residue

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
III	10	2	20.2
(7 rats)	12	4	20.2
	21	13	31.2
	21	13	35.0
	23	15	32.0
	27	19	31.7
	29	21	31.7

The preparatory period during which the animals received the deficiency diet and the period of medication were identical with those of litter I. At no time during the 21 days did any of these test animals show evidence of rickets.

For comparison with the foregoing experiment litter IV (table 6) was fed upon the mineral-deficiency diet for 8 days. Then for 21 days these animals received daily, in addition to the mineral-deficiency diet, 5 minims of a preparation (representing 1000 rat units of vitamin D) obtained by diluting 1 gm. 10,000 X viosterol to 25 gm. with maize oil.

Comparing tables 4, 5, 6 we find that ash content progressively increases in approximately identical fashion no matter whether the vitamin D is administered as an aqueous preparation (aquasterol F) or in oily solution (talc residue, viosterol dilution).

TABLE 6

Bone ash determinations: deficiency diet from date of weaning; medication by viosterol

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
IV (7 rats)	16	8	29.9
			26.9
			32.3
			33.5
	29	21	29.8
			30.0
			29.9 Ave. 30.4

The histological features of the tibiae studied on test animals from these three litters are practically identical. That is to say, restoration of the typical structure of healthy growing bone is facilitated by administration of vitamin D in any form. We shall have more to say about the mineralization of the bone itself.

A weaker preparation, aquasterol G, was obtained by 'extracting' 10 cc. of 10,000 X viosterol with water to a final volume of 250 cc. The two litters of rats (VI and VII) used in this experiment were maintained on the mineral-deficiency diet for 21 days from the date of weaning. From the twenty-second to the fifty-third day, inclusive (32 days in all), each rat received daily 1 cc. of aquasterol G by dropper. In this experiment the increase in skeletal ash-content (table 7) though small and variable, is nevertheless distinct. The

histological preparations demonstrate progressive restoration so that, at the end of the experiment, the bones of each surviving animal showed an approximately normal growth zone.

Figures 1 to 4 represent the histological changes in litters VI and VII which received the more dilute preparation (aquasterol G) after a preparatory period of 21 days on the mineral-deficiency diet. These figures, while illustrative of

TABLE 7

Bone ash determinations: deficiency diet from date of weaning; medication by aquasterol G

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
VI	21	0	29.5
(9 rats)	24	3	26.7
	29	8	28.7
	37	16	26.8
	37	16	25.6
	45	24	31.1
	45	24	29.1
	53	32	28.0
	53	32	32.8
VII	21	0	28.1
(8 rats)	33	12	30.6
	33	12	27.0
	37	16	28.6
	45	24	29.4
	45	24	31.4
	53	32	28.0
	53	32	34.5

the several other experiments reported in this communication, are particularly significant because they indicate what is really happening to a bone lacking in adequate mineral supply but under the influence of vitamin D.

In figure 1 the mineral-deficiency diet during 21 days has resulted in a generally defective mineralization quite characteristic in its features. The compacta is thin; the spongiosa is but partly mineralized so that there are areas of osteoid tissue interspersed among the bony trabeculae. This results

in a lattice-like architecture quite readily demonstrable by roentgenography. The distal margin of the spongiosa is irregular so that osteoid masses break the uniformity of its line. Beyond this is the zone of hypertrophic cartilage totally devoid of mineralization and even showing cystic areas. Beyond this again is the zone of proliferative cartilage also devoid of mineralization and indistinguishable from the diaphyso-epiphysial plane which appears unaffected. The bony epiphysis shows a demineralization comparable with that

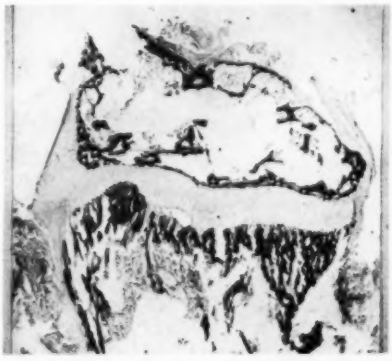

Fig. 1 Upper extremity tibia, negative control. Mineral-deficiency diet 21 days. No medication. Poor mineralization of compacta and spongiosa in both shaft and epiphysis. Areas of osteoid tissue interspersed between trabeculae of spongiosa. Cyst formation. Absence of mineralization in zones of hypertrophic and proliferative cartilage. Diaphyso-epiphysial plane apparently unaffected.

of the shaft but no other abnormal condition. It is true that this description differs from that usually presented in the literature. The difference is not in fact but in terms. We believe that an adequate anatomical analysis is essential to a proper interpretation of the phenomena accompanying experimental studies of dietetic deficiencies.

Figure 2 shows the change evident after 8 days of medication (1 cc. aquasterol G daily) during which the mineral-deficiency diet was continued. The compacta is thinner and the spongiosa more defective in mineralization. The area of

hypertrophic cartilage shows a mineralization of characteristic pattern (Dodds, '32) differing completely from the trabecular ossification of the spongiosa and capping the margin of the spongiosa by a uniform unbroken line of mineralization. The area of proliferative cartilage is not completely penetrated by mineral and therefore, although the clear area is narrowed it is not reduced to the thickness of the diaphyso-epiphysial plane which, with the adjacent tissue of

Fig. 2 Upper extremity tibia. Mineral-deficiency diet 29 days, supplemented during the last 8 days by 1 cc. of aqueous preparation of vitamin D (aquasterol G) daily. Compacta and spongiosa more poorly mineralized. Irregularity of spongiosa covered by unbroken cap of mineralization in zone of hypertrophic cartilage. Diaphyso-epiphysial plane remains unaffected.

the bony epiphysis, remains apparently unaffected. Of course demineralization is evident in the spongiosa of the epiphysis as in the spongiosa of the shaft.

Figure 3 illustrates the approximately normal healthy appearance of the growth area after 12 days of medication. Restoration has taken place at the expense of spongiosa in shaft and epiphysis which are more demineralized than before. The compacta is now quite defective in places; the spongiosa shows large areas devoid of mineralization and recognized as osteoid tissue. The zone of proliferative carti-

lage is almost wholly transformed into a zone of provisional calcification. There are no cystic areas and the march of mineralization toward the margin of the diaphyso-epiphyseal plane is clearly seen.

Figure 4 demonstrates the maintenance of a fairly healthy growth zone even after 53 days of the deficiency diet, on the last 32 of which the medication was given daily. Compacta and spongiosa are more defective than ever in mineralization.

Fig. 3 Upper extremity tibia. Deficiency diet 33 days, supplemented during the last 12 by 1 cc. aquasterol G daily. Compacta and spongiosa still more poorly mineralized. Zone of proliferative cartilage also partly mineralized so that clear area adjacent to diaphyso-epiphysial plane is reduced in thickness. Approximate restoration of normal growth area at expense of mineralization in epiphysis and shaft.

The partial mineralization of the zone of hypertrophic cartilage is now maintained with difficulty. The clear area comprising diaphyso-epiphyseal plane and adjacent zone of proliferative cartilage is now wider again owing to relative failure of penetration of the latter by the mineral salts. The uniformity of mineralization of the hypertrophic zone adjacent to the spongiosa is still maintained unbroken but it now forms merely a thin cap on the erratically ossified spongiosa.

This figure is particularly significant for it indicates the progressive constitutional strain incident to an attempt, by means of vitamin medication, to maintain a normal bony architecture despite the continuance of mineral deficiency.

Three litters (XII, XIII, XIV) consisting of fourteen animals were utilized in a test of aquasterol H made by 'extracting' with water an ordinary grade of commercial cod liver oil so that each cubic centimeter of the product contains the

Fig. 4 Upper extremity tibia. Deficiency diet 53 days, supplemented during the last 32 by 1 cc. aquasterol G daily. Very defective mineralization of compacta and spongiosa. Mineralization of proliferative cartilage zone lost and that of hypertrophic zone failing so that clear area adjacent to diaphyso-epiphysial plane is again widening.

water-soluble extractives of 1 cc. of cod liver oil. One cc. of this preparation was fed daily for 24 days to each of the four test animals in litter XIII after it had been subjected to a preparatory period of mineral-deficiency feeding lasting 27 days from the date of weaning. Litter XII was maintained on the deficiency diet for a period of 27 days after weaning but, thereafter, as a control to litter XIII, the deficiency diet was supplemented by 1 cc. daily of the stock cod liver oil from which aquasterol H was obtained. Litter XIV was main-

tained on the deficiency diet throughout the experimental period.

Histological studies of litter XII showed commencing restoration of the growth area after 4 days of medication (Bills et al., '31, p. 633) and approximately normal structure after 13 days. Litter XIII showed, at 13 days, a histological picture similar to that shown by litter XII at 4 days. It is evident then that the efficacy of aquasterol H was roughly one-third that of the cod liver oil. Ash determinations from these litters are recorded in tables 3, 9. Once again the histological picture is restored to an approximate normal by the water-soluble fraction of the antirachitic principle (vitamin D) even though the mineral of the skeletal depot is relatively deficient as shown by the bone-ash values.

For the second series of experiments an aqueous solution of crystalline activated ergosterol was obtained by dissolving 0.1 mg. of the crystalline substance in 250 cc. of water. One cc. of this preparation was fed daily to each test animal of litters IX, X, XI which were maintained upon the deficiency diet for 35 to 39 days after weaning and before administering the medication.

To judge from comparison with the figures from control animals segregated at the foot of the columns in table 8 mineralization of the skeleton is retained much better in the presence of vitamin D than without it. The histological pictures are comparable with those already described, and from them it is evident that medication administered for 6 days in this experiment restores in part the healthy appearance of the growth area while medication for 9 days approximately completes the restitution. Nevertheless vitamin D, though it directs the disposition of mineralization, cannot secure an actual increase of mineralization in the absence of an adequate supply. That the ash content of the bones in the protected animals was maintained indicates that just as vitamin D directs available calcium to the growth area so it must have some function in directing surplus calcium, wherever found, into the skeletal depots. This is probably what is meant by the assumed power of vitamin D to strengthen bones by in-

creasing their mineralization. Where the mobilization of this calcium takes place is not a problem for our present attention.

Maintenance of ash percentage also means that velocity of growth in the bone is not disproportionate to the available mineral during the 33 days of medication.

TABLE 8

Bone ash determinations: deficiency diet from date of weaning; medication by ergosterol

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
IX	28	0	35.7
(6 rats)	36	0	35.3
	36	0	32.8
	48	9	37.0
	70	31	34.7
	Control 70	0	31.24
X	28	0	29.6
(7 rats)	33	0	30.7
	45	6	29.4
	45	6	31.6
	64	28	28.9
	72	32	27.6
	Control 72	0	25.1
XI	1	0	31.8
(6 rats)	27	0	28.6
	32	0	29.7
	53	18	25.8
	73	33	32.3
	Control 73	0	26.8

TABLE 9

Bone ash determinations: deficiency diet from date of weaning; medication by aquasterol H (litter XIII) and cod liver oil (litter XII)

LITTER	DAYS AFTER WEANING	DAYS OF MEDICATION	PER CENT ASH
XII	31	4	33.5
(3 rats)	31	4	33.6
	40	13	34.4
XIII	33	6	30.6
(4 rats)	40	13	29.7
	43	16	30.9
	52	25	27.6

CONCLUSION

The practical conclusion to which these experiments point is that restoration of an approximately healthy growth area in the bones can be induced apart from replenishment of the skeletal mineral depots. A relatively minute water-soluble fraction obtained from cod liver oil or viosterol will remineralize and restore to a healthy appearance the growth area as effectively, though not necessarily with the same speed, as a relatively concentrated watery solution of crystalline activated ergosterol or as the oily preparations themselves. In bringing about this result vitamin D apparently utilizes mineral drawn from the shaft of the bones and possibly even from the body tissues.

Skeletal mineralization can be maintained only by an adequate mineral ration and an unimpaired mechanism of absorption and utilization. Skeletal demineralization will otherwise be progressive despite the administration of vitamin D.

SUMMARY

1. The administration of aqueous preparations (aquasterol) of the antirachitic principle to growing white rats maintains or restores an approximately normal histological pattern in the growth area of bones despite severe pathological disturbance induced by a mineral-deficiency diet.

2. Accompanying this morphological restitution there is a maintenance of skeletal ash percentage in the animals receiving medication, provided that the velocity of growth is not out of proportion to the available mineral. Control animals not receiving medication show a progressive diminution in skeletal ash-content despite the fact that the mineral content of the diet is undiminished.

3. Histologically, commencing restoration of the normal pattern can be seen as early as the fourth day of medication; maximum restoration is evident on the ninth day. Continuation of the experiment upon the growing animal results in a progressive skeletal demineralization despite the maintenance of an approximately normal histological structure in the growth area.

4. It is therefore apparent that restitution of the normal morphology in the growth area and replenishment of skeletal mineral depots are two separate though usually closely related phenomena.

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STUDIES ON THE ADRENAL

VII. THE RELATION OF THE ADRENAL CORTICAL HORMONE TO THE VITAMINS [1]

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FIVE FIGURES

(Received for publication March 29, 1934)

The idea of the existence of an intimate relationship between the adrenal cortex and certain vitamins (A, B (B_1), C and G (B_2)) has received increasing attention in recent years. This supposed relationship has been based upon: 1) the high concentrations of the vitamins (particularly A and C) occurring in the adrenal; 2) the changes occurring in the adrenal during avitaminosis (atrophy in avitaminosis A; hypertrophy in avitaminosis B and C); 3) the increase in susceptibility to infectious disease in avitaminosis and in adrenal insufficiency; 4) the similarity between certain of the clinical manifestations of avitaminosis and of adrenal insufficiency; 5) the alleged ameliorating effects of cortical extracts in avitaminosis B and C; 6) the similarity between certain of the chemical properties of the adrenal cortical hormone and vitamin G (B_2).

Of the above considerations, the first may be dismissed as a coincidence due to solubility factors which determine the distribution of a given substance in the body. Thus the high

[1] The results recorded here were presented before the Federation of American Societies for Experimental Biology at New York, March, 1934.

[2] Aided by grants from the National Research Council and the Josiah Macy, Jr., Foundation, for which we wish to express our appreciation.

concentration of carotene (pro-vitamin A) in the cortex may be attributed to the high lipoid content of the gland. Other lipoidal tissues, such as the corpus luteum (Huszak, '33) also contain equally high concentrations of pro-vitamin A, and we may therefore consider the existence of pro-vitamin A in the adrenal to be due to the laws of distribution based on simple solubility relationships. The adrenal atrophy occurring during avitaminosis A may be considered as part of a general reaction to the lack of a vital factor necessary for the well-being of many organs and tissues. There seems no reason, therefore, to assume an intimate relationship between vitamin A and the adrenal cortex, as Mitzkewitsch ('34) has also recently demonstrated.

In the case of vitamins B, C and G, however, the alleged ameliorating effects of adrenal cortical extracts in avitaminosis and similarities in the chemical properties of certain of these vitamins and the hormone, led us to reinvestigate the possible relationship between them.

VITAMIN B (B_1)

Pico-Estrada ('27) demonstrated the increased susceptibility of adrenalectomized rats to avitaminosis B. It is well known, however, that adrenalectomized animals are hypersensitive to any abnormal condition—the adminstration of drugs, dietary deficiencies, temperature extremes, excitement, et cetera—and hence his observations indicate no special relationship between the adrenals and vitamin B.

Schmitz and Kühnau ('33) and Lockwood and Hartman ('33) have claimed that they could ameliorate the symptoms of vitamin B deficiency by administration of cortical extracts. However, since the adrenal cortex is rich in vitamin B content, it is imperative to remove all traces of this vitamin before one can conclude that the effects observed after administering a given extract are due to the cortial hormone rather than to simple contamination by the vitamin. The cortical hormone as prepared by most current methods is a relatively crude extract containing a number of constituents.

When one considers how difficult it is to remove the last traces of vitamin B from relatively pure casein or from refined

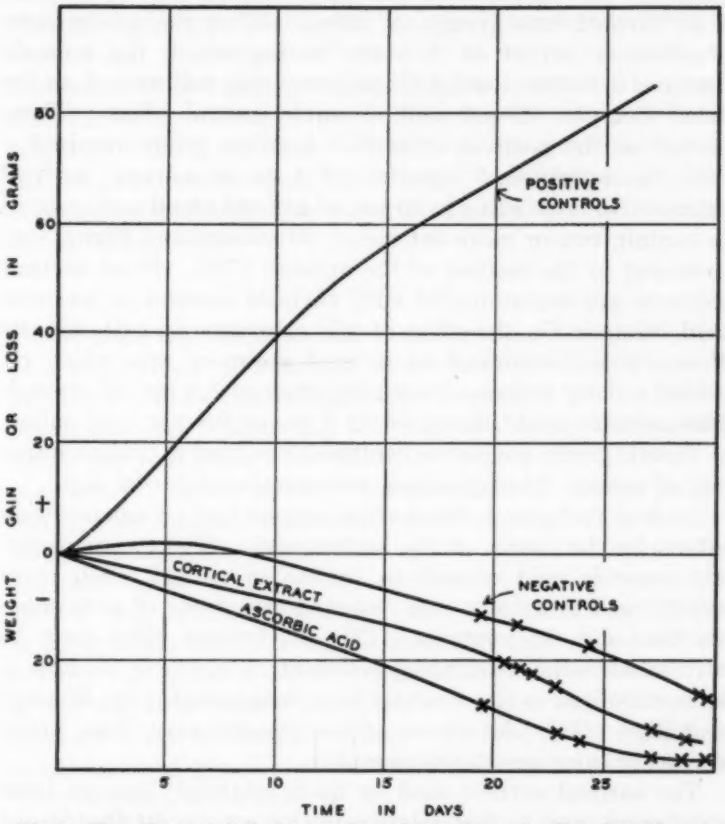

Fig. 1 The effects of daily intraperitoneal injections of saline (negative controls), adrenal cortical extract and ascorbic acid on the weight curve and survival of rats on a vitamin B-free diet. X indicates the death of an animal. The abscissae indicate the days elapsing after the preliminary deprivation period.

lactose one might suspect the contamination by vitamin B of comparatively crude cortical extracts.

Our experiments (reproduced in fig. 1) were carried out on 4-week-old rats matched as to sex, weight and litter and

placed on Chase's modification of Sherman and Spohn's diet (Sherman and Smith, '31). All the precautions described by Sherman and Smith were carefully observed. The animals were divided into groups of seven. After the preliminary deprivation period of 2 weeks during which the animals assumed a constant weight, one group was maintained on the basal diet plus 10 per cent of whole ground wheat. These served as the positive controls. Another group received a daily intraperitoneal injection of 1 cc. of adrenal cortical extract (corresponding to 50 gm. of adrenal gland and assayed to contain two or more rat units) (Grollman and Firor, '34) prepared by the method of the authors ('33). Since cortical extracts are contaminated with variable amount of ascorbic acid (vitamin C), the effect of this substance on avitaminosis B was also determined on a third group of rats which received a daily intraperitoneal injection of 0.5 mg. of crystalline ascorbic acid [3] dissolved in 1 cc. of 0.8 per cent saline. A fourth group (negative controls) received a similar quantity of saline. The injections were continued for 30 days.

As seen in figure 1, the cortical extract had no ameliorating effects on the course of the avitaminosis. The group receiving ascorbic acid seemed to decline in weight much more rapidly and to develop more marked symptoms of avitaminosis than did the controls. This deleterious effect may be attributed to the stimulating action of an excess of vitamin C on metabolism, as has recently been demonstrated by Mosonyi and Rigo ('33). An excess of one vitamin may, thus, prove decidedly injurious to the organism.

The cortical extract used by us is relatively free of inert substances, and to this relative purity can be attributed our results. The opposite effects of Schmitz and Kühnau ('33) and Lockwood and Hartman ('33) must, on the other hand,

[3] A portion of the ascorbic acid used in this work was generously supplied by Prof. A. v. Szent-Györgyi to whom we wish to express our thanks. The remainder of the material was in part prepared by us by the method of Svirbely and Szent-Györgyi ('33) and in part obtained commercially. All samples were the pure crystalline product corresponding to the empirical formula $C_6H_8O_6$ (Szent-Györgyi, '33).

be attributed to contamination by vitamin B derived from the original glandular material.

Considerable emphasis has been laid by previous writers (McCarrison, '21; Viale, '33) on the changes occurring in the adrenal in avitaminosis B. Gross and histological examination of the adrenals of our animals revealed no more striking changes than occurred in other endocrine glands and might have been anticipated from the inanition and retardation in growth accompanying vitamin B deficiency. In fatal cases of beriberi only minor changes in the adrenal have been noted (Hess, '20).

VITAMIN G (B_2)

The tests with this vitamin were carried out on 4-week-old rats maintained on Bourquin and Sherman's diet (Sherman and Smith, '31)—purified casein, 18 per cent; Osborne-Mendel (Sherman and Smith, '31) salt mixture, 4 per cent; butter fat, 8 per cent; cod liver oil, 2 per cent and cornstarch 68 per cent. Vitamin B (B_1) was supplied by adding to this mixture an alcoholic extract of whole wheat. After the preliminary depletion period, one group of animals (positive controls) were reared on the above diet to which was added 10 per cent of whole-milk powder. The other two groups of six animals were kept on the basal diet alone. One of these groups was given a daily intraperitoneal injection of 1 cc. of 0.8 per cent saline (negative controls), while the other received 1 cc. of cortical extract (derived from 50 gm. of whole adrenal glands) of the same potency as that used in the preceding experiments.

The animals used in the various groupings were litter mates matched for size and sex. All the precautions outlined by Sherman and Smith ('31) were rigidly observed.

The results of our experiment, reproduced in figure 2, show the absence of any effect of the cortical extracts in ameliorating the symptoms of avitaminosis G. Our cortical extracts are apparently free of vitamin G and the experiments of figure 2 show the absence of any beneficial effects of the adrenal cortical hormone in avitaminosis G.

VITAMIN C

The fact that the adrenal cortex has such a high content of ascorbic acid has been adduced (Szent-Györgyi, '33; Viale, '33) as indicative of more than a casual relationship

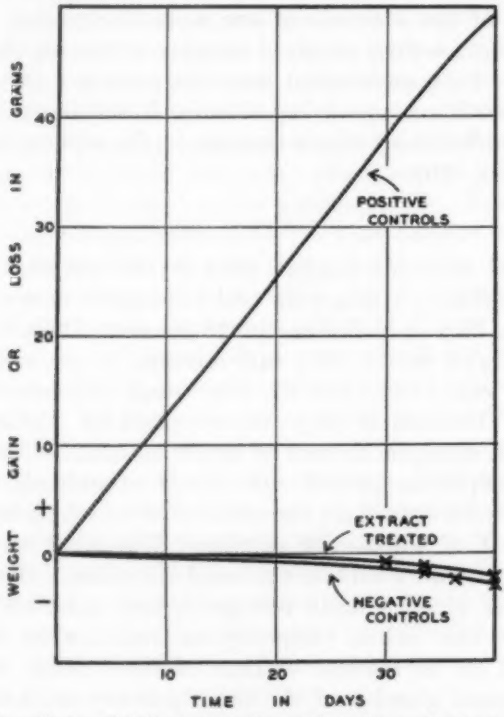

Fig. 2 The effects of daily intraperitoneal injections of adrenal cortical extract and saline (negative controls) on the weight curve and survival period of rats on a vitamin G-free diet. X indicates the death of an animal. The abscissae indicate the days elapsing after the preliminary deprivation period.

between the cortex and this vitamin. Grollman, Firor and Grollman ('34) have ascribed to this relationship a possible teleological significance for the close apposition of cortex and medulla in the adrenal.

The fact that vitamin C is present in such a high concentration in the adrenals renders it very difficult to prepare extracts of this gland which are free of the vitamin. The presence of ascorbic acid can be easily recognized in such extracts by spectrographic examination. In the cortical extracts prepared by the method of the authors ('33) relatively little ascorbic acid is present because of its elimination in the stage of preparation in which the cortical hormone is extracted from a neutral aqueous solution by benzol. The last traces of ascorbic acid may be easily removed by further purification. Such purified extracts were used in the following experiments. As a matter of fact, however, the amount of ascorbic acid present in our cortical extracts is less than that necessary to elicit any antiscorbutic activity, for essentially the same results were obtained when the unpurified cortical extract was used as were obtained after removal of the last traces of ascorbic acid. Spectographic examination demonstrated the absence of ascorbic acid in our purified cortical extracts.[4]

In conducting our tests for any antiscorbutic activity of the adrenal cortical hormone 6- to 8-weeks-old guninea pigs, weighing 300 to 350 gm. were placed on the scorbutic diet of Sherman, La Mer and Campbell (Sherman and Smith, '31). This consisted of rolled oats, 39 per cent; bran, 20 per cent; skimmed milk powder, heated in open trays at 110°, 30 per cent; fresh butter fat, 10 per cent, and NaCl, 1 per cent. The weight curves of the animals were followed until death, when the animals were autopsied and examined for the typical manifestations of scurvy. The experiments were conducted with four groups of four animals in each experiment. One group was given the basal diet plus lettuce (positive controls); a second group was maintained on the basal diet plus a daily injection of 2 cc. of 0.8 per cent saline (negative controls); the third group was given an intraperitoneal injection of 2 cc. of extract (derived from 100 gm. of adrenal glands) daily.

[4] We wish to express our thanks to Dr. Sterling B. Hendricks for these spectrographic analyses.

The experiment was repeated three times (total of thirty-six animals), but inasmuch as the results were identical in each case, only a single experiment need be reproduced here (fig. 3).

As seen in figure 3 the daily administration of extract derived from 100 gm. of adrenal glands manifested no antiscorbutic activity. This amount of extract corresponds roughly to several times that elaborated by the adrenal glands

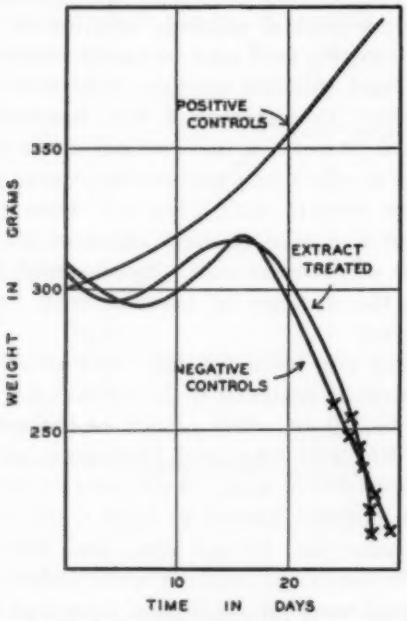

Fig. 3 The effects of daily intraperitoneal injections of adrenal cortical extract and saline (negative controls) on the weight curve and survival of guinea pigs on a scorbutic diet. X indicates the death of an animal.

of the normal guinea pig, as judged from its life-prolonging action in adrenalectomized animals, and should have been ample, therefore, to elicit any antiscorbutic activity. Autopsy of our experimental animals revealed the same degree of scurvy in our extract-treated animals (average scurvy score, 20) as in the negative controls (average scurvy score, 21) (Hess, '20; Sherman and Smith, '31).

Barbieri and Gambaro (quoted by Viale, '33) claimed that they could prolong the life of guinea pigs on a vitamin C-free diet with cortical extracts. Lockwood and Hartman ('33) in an extended study found that intraperitoneal injection of 'cortin' improved the growth curve in avitaminosis B and C, and ameliorated the symptoms when they occurred. Their results must be attributed to contamination of their extracts with ascorbic acid rather than to any effect of the cortical hormone itself, as they inferred.

In support of the view that the beneficial effects of 'cortin' in avitaminosis are not due to an admixture of ascorbic acid in their extracts, Lockwood and Hartman ('33) have advanced two arguments: 1) the insolubility of ascorbic acid in ether, which they used as their extraction medium, and, 2) the ineffectiveness of 'cortin' when administered orally. Their first argument can carry little weight, however, for a given solvent will, as is well known, extract substances from a gland which in a pure state would be insoluble in the solvent. Moreover, although relatively insoluble in ether the large volumes of the solvent used by these authors would dissolve an appreciable amount of ascorbic acid even in the pure form. Their second argument is based on another fallacy, namely, the assumption that the efficacy of ascorbic acid when administered orally is equal to that of the same amount of the substance injected parenterally. As a matter of fact, a priori considerations based on the chemical properties of ascorbic acid would lead one to suspect that its oral administration would require, as is the case with many therapeutic agents, larger doses than does intraperitoneal administration. Hence, the injection of a very small dose (present as an admixture in crude adrenal extracts) intraperitoneally might lead to an amelioration of the scorbutic symptoms which would not be detected after oral administration. The experimental demonstration of the correctness of this view is reproduced in figure 4.

Groups of twelve guinea pigs were maintained on the scorbutic diet described above. Besides the usual positive (lettuce

added to diet) and negative controls, one group was injected daily with ¼ mg. of ascorbic acid dissolved in ½ cc. of 0.8 per cent saline, while the second received an equal quantity of the vitamin, orally. The curves of figure 4 show the greater therapeutic activity of the parenterally administered hormone. Whereas all of the animals treated orally died during

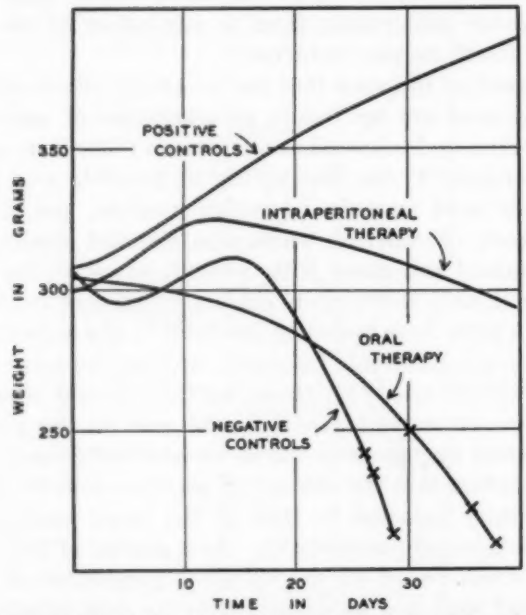

Fig. 4 A comparison of the effects of the oral and intraperitoneal administration of ¼ mg. of crystalline ascorbic acid daily on the weight curve of guinea pigs on a scorbutic diet. X indicates the death of an animal.

the experimental period with marked scurvy (average scurvy score, 18); those injected intraperitoneally with the same amount of material showed prolonged survival and only minor degrees of scurvy at autopsy (average scurvy score, 9). This is essentially the same result obtained by Lockwood and Hartman in their study of the comparative effects of the oral and intraperitoneal administration of cortical extract and we must

conclude that their results are due to the relative efficacy of different modes of administration. Their extracts apparently contained about ¼ mg. of ascorbic acid in 2 cc. (their dosage) which, as we have seen, is not unexpected.

Considerable emphasis has recently been placed on the hypertrophy and abnormalities occurring in the adrenal during avitaminosis C. It is questionable how much of the observed hypertrophy is due to the starvation accompanying scurvy and to concurrent infections, which are common, and both of which, as Hess ('20) has pointed out, complicate the picture. It is significant that in human scurvy there are no marked alterations in the adrenal (Hess, '20). In our own animals we have frequently observed hemorrhage of the adrenals similar to that which is found in the muscles, gastrointestinal tract, gums, et cetera, and which, therefore, is not a specifically adrenal manifestation. The medulla often presents a grossly altered appearance being converted to a soft, chocolate-colored, hemorrhagic mass.

THE EFFECT OF ASCORBIC ACID IN ADRENALECTOMIZED RATS

Because of the close association between ascorbic acid (vitamin C) and the adrenal cortex, it seemed desirable to test the effects of this substance on adrenalectomized rats. The results of a typical experiment are given in figure 5. The upper curve shows the effect of administering the cortical extract prepared as described by us (Grollman and Firor, '33) to bilaterally adrenalectomized rats. When the animals are treated with a potent extract as in the upper curve of figure 5, they grow normally [5] until several days after cessation of treatment, after which they rapidly lose weight and die. Untreated controls, as shown in the lowest curve, fail to gain weight and die in a much shorter period of time than the treated animals.

[5] The results of Gaunt and Gaunt ('34), which led them to question the validity of the rat method for the assay of the adrenal cortical hormone, are due to faulty operative technic, as previous workers and we (Grollman and Fioror, '34) have demonstrated. The results of figure 5 illustrate the adequacy of our method of assay.

As seen in the middle curve of figure 5, the daily administration of 1 mg. of ascorbic acid intraperitoneally has no demonstrable effect on the survival or weight curve of adrenalectomized rats. This observation is in accord with the statement of Szent-Györgyi ('33) that ascorbic acid has no beneficial effect in patients suffering from Addison's disease. This observer states, however, that administration of vita-

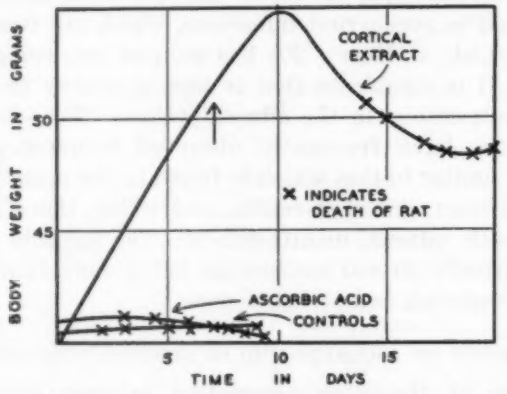

Fig. 5 An assay illustrating the absence of any effect of ascorbic acid on the weight curve and survival of adrenalectomized rats. The upper curve shows the response of the operated animals to the administration of adrenal cortical extracts for 7 days. The lowest curve (controls) shows the response to saline injections. All operations were performed at one sitting under amytal anesthesia. X indicates the death of an animal. The arrow indicates the day on which the injections were discontinued.

min C removes the bronzing of such patients. Accordingly, we must consider this important clinical feature of Addison's disease as being only a secondary result of disease of the adrenal cortex and not due to insufficiency of the cortical hormone itself. The occurrence of the typical clinical picture of Addison's disease without pigmentation (Ghrist and Rowntree, '27) may be explained on the basis of the above considerations.

DISCUSSION

The above experiments are evidence against the view expressed by many previous authors that there exists a particularly intimate relation between the vitamins and the adrenals. The susceptibility of the adrenal gland to conditions of avitaminosis is not unique, for other endocrine organs (thyroid, pituitary, sex glands, etc.) are equally affected (McCarrison, '21) by dietary deficiencies. The vitamins, apparently, are essential for the well-being of a great number, if not all, of the organs and tissues of the body. The similarity between certain clinical manifestations of dietary deficiencies and of adrenal insufficiency are due to the fact that the cortical hormone is also necessary for the well-being of a number of tissues and organs. Hence, deficiency due to either vitamin or hormone leads to a multiplicity of manifestations (in the gastro-intestinal, nervous, cardiovascular, endocrine and other systems) and to a superficially similar symptomatology. The increased susceptibility to infectious disease in avitaminosis and in adrenal insufficiency is only an indication of a general lowering of body-resistance—a condition occasioned by a number of causes.

SUMMARY

The adrenal cortical hormone was found to have no demonstrable replacement activity in experimental avitaminosis B or G.

The apparent partial replaceability of vitamin C by adrenal cortical preparations reported by previous observers is due to the presence of ascorbic acid in such extracts. Crystalline vitamin C is more effective when administered intraperitoneally than by mouth. Ascorbic acid does not prolong the life of adrenalectomized animals.

The other considerations which have led previous observers to assume the existence of an intimate relation between the adrenal cortex and vitamin deficiencies are explicable on the assumption that the hormone and vitamins are necessary for the proper functioning of a great number of organs and tissues.

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THE EFFECT OF SUPPLEMENTARY IODINE ON THE NUTRITIVE VALUE OF CHICK RATIONS

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FOUR FIGURES

(Received for publication April 9, 1934)

As early as 1851, Chatin (1851) observed that the prevalence of goiter varied with the iodine content of the soil of different localities. The subsequent extensive studies of the factors which influence the development of human goiter confirm this observation. Furthermore, chemical analyses of numerous fruits, vegetables, milk, etc., by Forbes and coworkers ('16), McClendon and associates ('30), Remington ('30) and others show a wide variation in iodine content depending upon the iodine content of the soils upon which the materials were produced.

Numerous investigators have shown that a continued deficiency of iodine in the rations of farm animals produces various abnormal conditions. Welch ('28) observed goiter in foals, calves, lambs, pigs, goats and dogs. He says, "Goiter of the adult animal, as compared with goiter of the new-born, is relatively rare." He also states that the loss of young pigs "was so great that swine breeding was practically abandoned in many sections of Eastern Montana and Western North Dakota," but the use of iodine as a prophylactic agent eliminated the difficulty. Shepperd ('29) and others have associated deficiency of iodine intake with the hairless pig malady. Fenger and associates ('31) found the iodine content of the thyroid gland (hog) varied with the geographical

location from which the animals came. Kalkus ('20) says, "It is variously estimated by stockmen that from 75 to 90 per cent of the colts in some of these goiterous districts are affected with the trouble, and that 95 per cent of such animals are either still-born or die two or three days later. The remaining 5 per cent never make perfect recovery." Hudson ('33), Harvey ('29), Cooley ('31), Rodenwold and Simms ('34) and others have reduced the prevalence of navel disease or joint ill in newborn foals by feeding potassium iodide to mares during pregnancy. Several investigators have reported that administration of iodine influences reproduction in farm animals. For instance, Phillips, Curtis and Erf ('34) say "cows of the herd previously sterile have become pregnant and borne normal calves during this regimen of feeding." Marine and Kimball ('21) state that sodium iodide has been successfully used on a large scale in the prevention of goiter in cattle, sheep, pigs and poultry. Weiser and Véghelyi ('32) report that merino sheep, of an iodine deficient region, when fed iodine regularly produced larger lambs and 10 per cent more wool than a control flock.

These varied observations concerning the effect of an inadequate supply of iodine on the health of hogs, horses, cattle and sheep naturally raise a question as to whether commercial poultry rations contain an adequate supply of iodine. The cereal grains which constitute a larger portion of commercial poultry rations are grown on soils far removed from the ocean and of reputed low iodine content. Numerous analyses by Forbes and co-workers ('32) show that corn, oats and wheat rarely contain more than a 'trace' of iodine and the majority of the samples contained 'none.' Welch ('28) reports "Goiter in poultry is very common. Flocks with a very high percentage of it have been noted in goiterous areas." On the other hand, Kernkamp ('25), who has made a detailed report of simple colloid goiter in poultry states that goiter in poultry is very uncommon.

Hercus and Roberts ('27), Simpson and Strand ('30), Jaschik and Kieselbach ('31), Scharrer and Schropp ('32),

Schmidt ('32), Zickgraf ('32), Straub ('33), and Wilder, Bethke and Record ('33) increased the iodine content of eggs by supplementing the ration of laying hens with various iodine compounds. Also Scharrer and Schropp ('32) and Klein ('33) found that supplemental iodine increased egg production, but Malan ('31) obtained the same egg yield for both experimental and control birds. The results that were observed when the rations of adult birds were supplemented with iodine suggested the possibility of enhancing the nutritive value of rations for growing chicks by supplementing them with iodine compounds. The present investigation was conducted to obtain data on this point, but no attempt was made to accumulate data concerning the effects of iodine deficiency or concerning the iodine requirement of growing chicks.

EXPERIMENTAL

The experimental mashes were prepared on a commercial scale in a thoroughly modern feed manufacturing plant. The basal mash was designed to include an adequate amount and a satisfactory proportion of the constituents essential for rapid, balanced growth of young chicks. It contained corn meal (attrition) 32 per cent, wheat bran 15 per cent, wheat flour middlings 15 per cent, ground oat groats 12 per cent, alfalfa leaf meal 5 per cent, dry skim milk 8 per cent, meat scraps 5 per cent, fish meal 5 per cent, sardine oil 0.5 per cent, dicalcium phosphate 0.5 per cent, oyster shell meal 1 per cent and salt 1 per cent. On analysis this ration was found to contain: moisture 9.75 per cent, protein 19.35 per cent, fat 5.50 per cent, fiber 4.30 per cent, carbohydrates 57.35 per cent, ash 9.10 per cent, calcium 1.57 per cent, phosphorus 1.03 per cent and approximately 500 parts of iodine per billion parts of mash. The basal mash was divided into five lots. One lot was retained as 'control' mash and potassium iodide was added to the other lots at the rate of 18.8 mg., 37.5 mg., 75.0 mg. and 93.8 mg. per kilogram of mash. The potassium iodide was first mixed with the salt. These were combined with the other mineral constituents of the ration and this

in turn was carefully incorporated into the ration. The mashes under consideration were assigned as follows:

Pen A—Basal mash
Pen B—Basal mash plus 18.8 mg. KI per kilogram
Pen C—Basal mash plus 37.5 mg. KI per kilogram
Pen D—Basal mash plus 75.0 mg. KI per kilogram
Pen E—Basal mash plus 93.8 mg. KI per kilogram

Rhode Island Red baby chicks purchased from a hatchery which maintained high producing, bacillary white diarrhea free stock served as subjects of this investigation. The parent stock was fed a combination of 'laying mash' and scratch grains. Data concerning the iodine intake of the parent stock are not available. Analysis of 'laying mash' manufactured in the same mill and composed of similar ingredients showed an iodine content of 604 parts per billion. Practical experience indicates that the amount of iodine supplied by a combination of this type of 'laying mash' and scratch grains meets the requirements of breeding stock. More definite information in this connection would be of interest, since Corrie ('33) reports that iodine deficiency in the ration of the laying hen causes a lowered resistance to disease in the progeny, and Weiser and Zaitschek ('32) state that the development of pigs is advantageously influenced by feeding iodine to pregnant sows. On the other hand, Scharrer and Schropp ('32) compared the results obtained in two groups of laying White Leghorn hens, one of which received KI as a supplement to its ration, and found "Growth of the chicks was equally good in both groups."

In general, the experimental procedure was similar to that employed in previous studies (Holmes et al., '33). The chicks were housed in all-metal battery brooders equipped with automatically controlled, electrically heated hovers. The windows of the battery room were covered and throughout the experiment all light was obtained from two 200-watt ceiling lamps which were surrounded with shields known as Red Laco Color Lights. The chicks were divided into five comparable groups of thirty chicks. From hatching until 12 weeks

of age the chicks received only the experimental mashes and drinking water, both of which were continuously before the chicks throughout the entire experimental period.

The chicks were weighed once a week. The average per chick weights for the cockerels and pullets are reported as

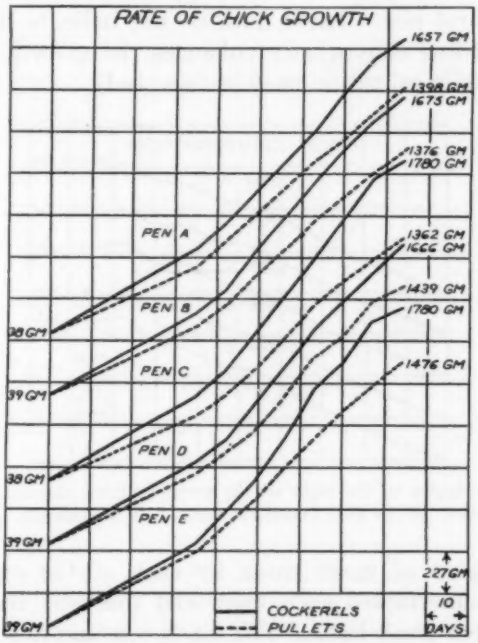

Fig. 1 The figures at the left of the growth curves indicate the initial weight of the baby chicks. The figures at the right indicate average per chick weight for cockerels and pullets at the termination of the experiment when the chicks were 12 weeks old.

growth curves in figure 1. The rate of growth of the chicks in the different pens was quite uniform and consistent during the experimental period. At 12 weeks of age the weight of the cockerels was 1657, 1675, 1780, 1666 and 1780 gm. and that of the pullets was 1398, 1376, 1362, 1439 and 1476 gm., respectively, for pens A, B, C, D and E. These weights show

that the growth of the chicks in all the pens was excellent. The weights of the cockerels of pens C and E are greater than that of the control pen. Likewise the weights of the pullets of pens D and E are higher than that of the control pen. On the other hand, the weight of the pullets in pens B and C, which received iodine supplements, is less than that of the control pen. These data do not indicate that supplementary iodine consistently enhances the growth promoting value of the basal ration used in this study.

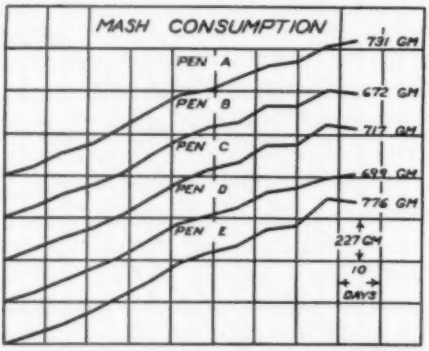

Fig. 2 The figures at the right of the curves indicate the average per chick mash consumption for the final (twelfth) week of the experiment.

The amount of mash eaten by each of the experimental pens was determined each week and this was reduced to a per chick per week basis. The mash consumption curves for the various experimental pens appear in figure 2. From these it will be noted that the amount of mash consumed per bird increased consistently and quite uniformly during the entire experimental period. The total mash consumed per bird for the entire experimental period was 5080, 4920, 5080, 4920 and 5260 gm. for pens A, B, C, D and E, respectively. Inasmuch as pen E, which received the highest amount of iodine, consumed the largest amount of mash it is evident that the continued ingestion of this amount of iodine did not decrease its palatability.

The nutritive efficiency of the five mashes under consideration has been computed on the basis of the amount of mash required to produce 100 gm. gain in weight. Such computations have been made at the close of each week of the test period. These data for the five mashes have been assembled as curves which appear in figure 3. The figures which appear

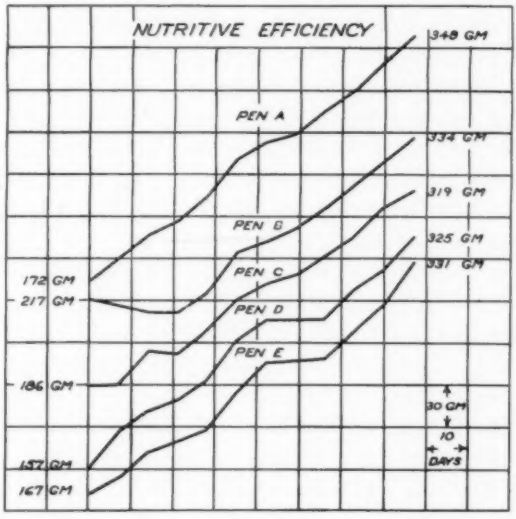

Fig. 3 The figures at the left of the curves indicate the amount of mash required to produce 100 gm. gain in body weight during the first week and those at the right indicate the amount required per 100 gm. of gain in weight for the entire 12-week period. The least amount of mash required to produce 100 gm. gain in weight was for pen C, hence mash C had the highest nutritive efficiency.

at the right of the curves represent the efficiency of the mashes for the entire 12-week period. Since the efficiency of the mashes is in inverse ratio to the amount consumed, mash C, which required 319 gm. to produce 100 gm. gain in weight, was the most efficient and mash A, which required 348 gm. to produce 100 gm. gain, was least efficient. If attention were confined to the results obtained with pens A, B and C, one would conclude that the nutritive efficiency of the control mash

increased as the amount of supplementary iodine was increased. However, the amount of iodine contained in ration D is twice that of ration C and yet the nutritive efficiency is lower. In the same manner mash E has five times the supplementary iodine present in mash B and the nutritive efficiency is essentially the same.

The physical appearance of the chicks (every bird) was carefully observed each week of the experiment. Attention was directed to the extent of feathering, the number of pinfeathers, the color of the shanks and beaks, the extent of development and the color of comb and wattles, and the general uniformity and alertness of the groups as a whole. Judged by these characteristics there was no significant difference in the physical appearance of the birds of the five pens under consideration.

The bone development of the chicks was judged by observations made on tibiae removed from five typical test chicks of each pen at the end of the 12-week period. The left tibiae were freed of tissue as soon as they had been removed from the birds, dried at 100°C. for 48 hours. They were then measured and weighed individually. These data were averaged for each pen. The dried left tibiae were ground and extracted with 95 per cent alcohol for 24 hours. For this purpose the entire tibia including the cartilage at the proximal end was used. The combined, dried, extracted tibiae of the five test chicks were incinerated to determine the ash content. The data for length, diameter, weight and ash content of the tibiae will be found in table 1. The length and diameter of the tibiae was least for the control pen and greatest for pen E, which received the largest amount of iodine. On the other hand, the average weight per tibia was greatest for pen B, which received the least amount of supplementary KI, and the tibia ash content was highest for pen C, which received one of the lower levels of KI. In view of these inconsistent results for the length, diameter and weight of the tibiae and for their ash content, it is questionable whether the addition of KI significantly improved the bone building value of the control ration.

When the right tibiae were removed from the test chicks they were carefully freed of all tissue and placed in formaldehyde for a number of days. Subsequently they were split, washed, stained with a 2 per cent silver nitrate solution, intensified under a bright light and photographed. The accompanying photograph (fig. 4) of a section of a tibia from a typical chick of each pen shows the internal structure of the tibiae from chicks which received the basal ration and the basal ration supplemented with different amounts of potassium iodide. Examination under a binocular microscope (thirteen diameters) of the tibiae sections from the five chicks of each experimental pen did not reveal any difference in the development of the tibiae of chicks which received supplementary KI from that of the control chicks.

TABLE 1

The tibia growth of experimental chicks
(12-week-old chicks)

PEN	LENGTH	DIAMETER	WEIGHT	ASH
	cm.	*cm.*	*gm.*	*Per cent*
A	11.67	0.75	8.11	46.89
B	11.93	0.90	9.01	47.40
C	12.00	0.88	8.71	48.85
D	12.14	0.88	8.33	48.11
E	12.30	0.95	8.91	47.30

Hemoglobin determinations were made of the blood of typical chicks, three cockerels and four pullets from each pen, at the termination of the experimental period. The determinations were made by the procedure previously described (Holmes et al., '33). The results obtained are reported in table 2. It will be noted that the hemoglobin content of the blood was highest for the control pen, pen A. While the hemoglobin values for the chicks which received supplementary iodine increased with the amount of iodine fed, they did not equal that of the control pen. These data show that the use of supplementary iodine did not increase the hematopoietic value of the basal ration. In fact, one might conclude that it tended to decrease the hematopoietic value of the control mash.

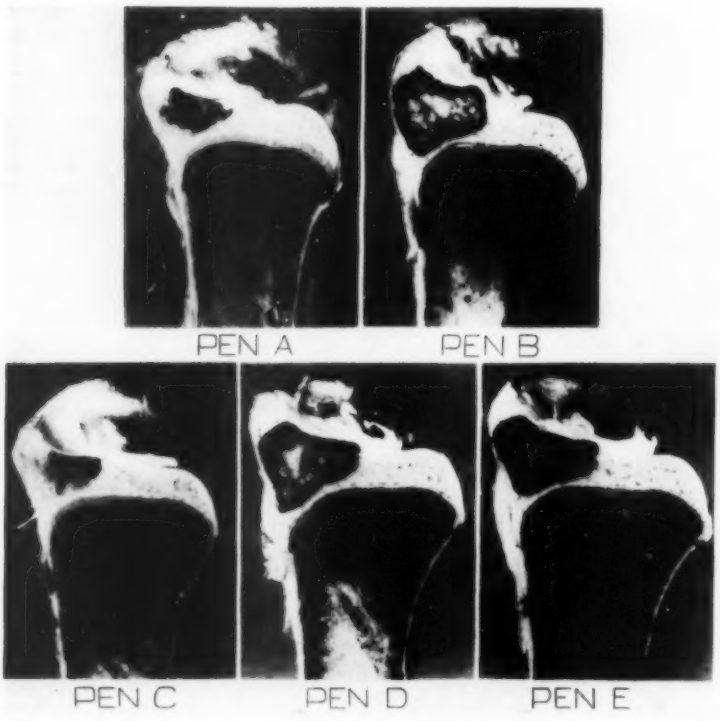

Fig. 4 Photographs of sections of split right tibiae of 12-week-old chicks. No significant difference can be detected in the extent of calcification of the tibiae of chicks from the different pens.

TABLE 2

Hemoglobin content of chicken blood
(12-week-old chicks)

PEN	COCKERELS	PULLETS	AVERAGE [1]
	(Gm. Hb. per 100 cc. of blood)		
A	10.37	10.03	10.24
B	9.02	9.45	9.26
C	9.22	9.54	9.37
D	10.04	9.93	9.96
E	9.96	10.31	10.16

[1] Weighted average.

The data which were obtained in this investigation are in accord with those reported by other investigators. In a carefully controlled experiment conducted by Hamilton and Kick ('30) "no evidence was obtained that a supplement of potassium iodide at the rate of 0.5 mg. or of 1.0 mg. daily per 100 gm. of body weight in any way influenced the rate of growth." Forbes and associates ('32) studied the influence of iodine on the growth of White Leghorn pullets (701). Groups 1 and 2 received the same mash and groups 3 and 4 another mash of different nutritive value. Groups 2 and 3 also received a sufficient amount of iodized linseed meal "to provide 50 mg. of iodine per 100 pounds of chicken a day." When 12 weeks old, the chicks of the control groups weighed more than the corresponding groups which received the iodine supplement to their ration.

SUMMARY

Five pens of thirty Rhode Island Red chicks were fed a high quality chick growing mash from hatching to 12 weeks of age under standardized laboratory conditions. The mash for four pens was supplemented with 18.8 mg., 37.5 mg., 75.0 mg. and 93.8 mg. of KI per kilogram of mash.

Observations were made on the growth, physical appearance and mash consumption of the chicks; the nutritive efficiency of the mashes; the bone growth and the hemoglobin content of the blood when the chicks were 12 weeks of age.

The results obtained were somewhat irregular. The final weight of the chicks did not vary consistently with the amount of iodine fed. The mash consumption was greatest for the pen receiving the most KI. The physical appearance was similar for all pens. The nutritive efficiency for mash C, to which was added 37.5 mg. of KI, was higher than that of the mashes which contained more or less KI. The length and diameter was greatest for tibiae from the chicks that received the most KI. On the other hand, the heaviest tibiae were from pen B and the ash content was larger for chicks from pen C than for those chicks which received more or

less KI. The internal structure of the tibiae showed the same degree of development for all pens. The hemoglobin content of the blood of the 12-week-old chicks was greatest for the control pen.

The variations in the results obtained for the different experimental pens with respect to growth, mash consumption, physical appearance of the birds, nutritive efficiency of the mashes, the size and composition of the bones, the internal structure of the tibiae, and the hemoglobin content of the blood do not appear to be highly significant, since these variations are not materially larger than might occur for two 'control' pens maintained under comparable conditions.

These results show that the amounts of KI fed as a supplement to the control ration did not significantly enhance its nutritive value. Since the chicks which received the control ration grew exceptionally well and showed satisfactory development in all respects, it would appear that the control ration contained an adequate supply of iodine to meet the needs of rapidly growing chicks.

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A NEW TOXICANT OCCURRING NATURALLY IN CERTAIN SAMPLES OF PLANT FOODSTUFFS [1, 2]

I. RESULTS OBTAINED IN PRELIMINARY FEEDING TRIALS

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EIGHT FIGURES

For many years livestock producers in certain localities have complained of unsatisfactory growth and reproduction in domestic animals. As early as 1857, Madison (1860), of the United States Army, noted a severe pathological disturbance among the cavalry horses of his post. The conditions which he described appear to be similar to, if not identical with, the observations which initiated the investigation begun in this laboratory in 1928.

As a result of feeding experience over a number of years, some farmers learned to associate the disturbance with particular tracts of land, although they had various opinions regarding the precise nature of the factors involved. Many believed that the toxic effects were due solely to drinking water, while others attributed the toxic effects to plants growing on their land. Samples of grain from farmers whose livestock had shown pathological symptoms exerted extremely

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[2] These investigations are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics of the United States Department of Agriculture.

toxic effects upon white rats. The grain samples were not diseased in any way and macroscopic examination revealed no abnormalities. The grain has no characteristic odor or taste. Routine feed analyses failed to reveal differences between affected and unaffected grains. Only by bioassay could the toxicity of the grain sample be determined.

The effects produced by feeding these toxic grains are apparently not duplicated by any known plant toxins such as the glucosides, toxalbumins, saponins, or alkaloids, nor can the results obtained be attributed to decomposed or contaminated foods. Furthermore, the deaths observed cannot be due to a deficiency of proteins, carbohydrates, fats, minerals, or vitamins. Experimental work indicates that the grains contain a definite toxic compound or compounds. The fact that these toxic effects are produced by grains which have an important place in the diets of both man and animal makes the question of tremendous significance.

In 1931 the results of the experimental work in this laboratory were brought to the attention of the United States Department of Agriculture. As a result of collaborative work, Robinson ('33) reported the occurrence of selenium in wheat and soil samples from affected areas, and Byers ('34) has reported chromium, vanadium, and arsenic in addition to selenium in soil samples from the same region. He also reports the occurrence of selenium and vanadium in a sample of wheat from the same locality. Nelson, Hurd-Karrer, and Robinson ('33) have questioned the use of selenium compounds in insecticides. They found that when selenium was added to soil in even minute quantities, the element was absorbed by wheat plants with the production of a compound extremely toxic to rats and guninea pigs.

A large number of feeding investigations have been conducted in this laboratory during the past several years. Concomitantly, studies of the gross pathology, the physiology, action on enzymes, and chemical nature of the toxic factor are being carried on. As the present paper is of the nature of a preliminary report, the effects produced by toxic grain samples on experimental animals will be stressed.

The experimental animal used in this investigation is the albino rat (M. norvegicus albinus) from stock obtained from The Wistar Institute of Anatomy and Biology. It has been found that not only do the rats exhibit wide variations in degree of tolerance, but affected grain samples show all degrees of potency. As the result of many feeding trials, the grain samples have been grouped into two types: lethal and sublethal. The conditions produced in rats by these foodstuffs vary from very severe effects, in which death occurred by the eighth day, to conditions in which growth retardation was the only observed effect. In all the work it has been found necessary to use a rigid control system.

The resistance of the rats to the toxicity was increased by the use of various adjuvants. However, it must be borne in mind that in cases where rats receiving adjuvants showed less pronounced effects, the animals were receiving less of the toxicant per unit of total food consumption. It may be well to state here that the grains from which the following data were obtained were not treated chemically in any way, being merely ground before they were incorporated into various diets.

Incidence of death

By using the thirteen most potent samples of grain (lethal), death was produced in 321 out of 325 rats placed on experiment at various times in the past 4 years. The grains used included corn, wheat, barley, and emmer. Death occurred as follows: 16.3 per cent on or before 25th day of experiment; 38.5 per cent on or before 40th day; 71.1 per cent on or before 60th day; 92.0 per cent on or before 100th day. The largest percentage of the rats (35.7 per cent) died between the 34th and 54th days; 25.2 per cent died between 14th and 34th days, and 16.0 per cent died between the 54th and 74th days of experiment (fig. A).

Six other lots of grain (sublethal) gave very interesting results. Death occurred in only six of fifty-two rats by the sixtieth day. Autopsies on all animals, including those sacri-

ficed, revealed liver lesions which were very striking. Numerous other samples of grain, which do not cause death and may not cause macroscopic lesions, have been encountered. In these samples the only observed effect is depression of growth.

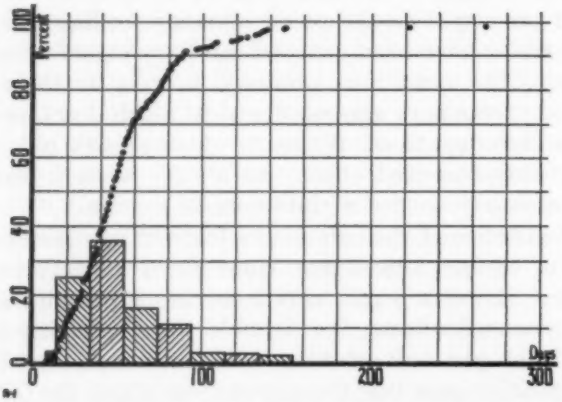

Fig. A Incidence of death in 325 rats on thirteen lethal grain samples. The points show the per cent of the total number of rats which were dead by the corresponding day, and the bars show the percentage of deaths in the indicated 20-day periods. The greatest number of deaths occurred between the thirty-fourth and the fifty-fourth days.

Symptoms of pathology

The symptoms and pathology to be described below are based upon observations and post-mortems on the rats which were used in the feeding trials.

When weaned rats are placed on a diet containing a high percentage of a lethal grain sample they respond immediately by restricting their food consumption to as low as 25 per cent of normal. Some rats lose weight rapidly; others gain weight slowly. Franke and Potter ('34) have shown that the pathology found in the affected animals is not due to inanition, because rats fed identical amounts of control grain show no abnormalities other than stunted growth.

The symptoms appear in a wide variety of forms and it is impossible to describe a definite syndrome which will cover all

cases. Most of the rats soon assume a characteristic hunched posture, and walk in the same position. The fur becomes rough and is usually stained a brilliant yellow around the genitals, due to excessive amounts of bile pigment in the urine. In a few cases the hind legs of the rats become completely paralyzed. In these animals, autopsies usually reveal hemorrhages into the subcutaneous tissues and the muscle fascia near the joints. Figure 1 shows the external appearance at death in a typical case from series 1.

In the first animals that die, the gross pathology is not marked, in comparison with later cases. The most apparent feature is the dilation of the veins in the visceral region. The vena cava and right auricle are almost invariably engorged.

Fig. 1 Appearance of rat no. 58F at death, after 131 days on diet containing lethal corn no. 388 (series 1).

The lungs and the liver usually have a congested appearance. The thymus is greatly degenerated. The reproductive organs are underdeveloped and may show degeneration. The stomach usually has a scanty black residue in it and the small intestines also show evidence of hemorrhage. The animals are usually jaundiced and the bladder is frequently distended with highly colored urine. The animals are always extremely emaciated. The cases just described will be referred to as subacute.

The remainder of the rats which die on lethal samples may well be referred to as chronic cases. Although they frequently show moderate increases in weight and may appear

to be in fairly good condition, a definite breakdown of vital organs takes place. These animals develop an anemia of progressive severity and finally die with hemoglobin levels as low as 2.1 gm. per 100 cc. Autopsies reveal striking pathology. The most outstanding feature is in the liver, which is atrophied, necrotic, and hemorrhagic in varying degrees. Figure 2 illustrates a case in which the macroscopic changes in the liver are but slight.

The toxicant evidently produces a definite necrosis of the hepatic cells. Animals with high tolerance are able to sur-

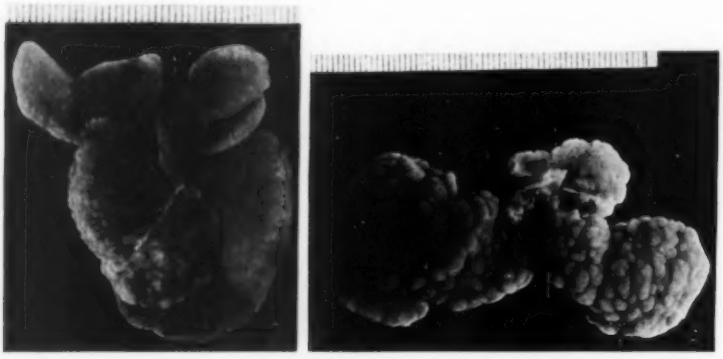

Fig. 2 Liver from rat no. A72F, which was killed after 153 days on a diet containing sublethal corn no. 594 (series 88).

Fig. 3 Liver from rat no. 1885M, which was killed after 365 days on a diet containing lethal wheat no. 582 (series 78).

vive for a longer period of time, because they are able to regenerate their liver cells almost as rapidly as necrosis occurs. It is usually found in regenerative cases that the hypertrophy occurs in either or both the right lateral and the caudate lobes. The left lateral lobe and the central lobes show terrific atrophy, the latter showing the most profound effects. An example of the regenerative type of liver is shown in figure 3, in which the caudate lobes show marked hypertorophy.

The chronic cases also show enlargement of the heart and spleen in many cases. The animals may or may not be jaundiced. Bone conditions resembling vitamin D deficiency

are often found, although the diets have always been adequate in that respect and the controls have developed normally. The bone condition may be analogous to that described by Buchbinder and Kern ('27). The thymus and reproductive organs are affected as in sub-acute cases. The lymph nodes are invariably enlarged and red. The intestinal contents are frequently slate-blue in color. Many of the rats become oedemic. Ascites and pleural oedema are also common. It is felt that the liver is probably one of the primary foci of the toxicant and that the majority of the symptoms are secondary effects. Further evidence for this is found in the fact that in many of the rats which have abundantly regenerative livers, there is very little other pathology. A number of chronic cases have died as a result of an internal hemorrhage from the liver.

The pathology observed in rats on sublethal grain samples is also very interesting. Sub-acute cases are almost never found and the rats which die usually show chronic symptoms.

An interesting case of liver regeneration was shown by a rat on a lethal sample of grain, plus liberal amounts of yeast, orange juice, and cod liver oil (series 20)—(fig. 4).

EXPERIMENTAL

It seems desirable to record the details of at least one experiment in this paper. Series 1 was carried out with a lethal sample of corn. Many grain samples show a higher degree of potency than the lot used in this experiment.

An examination of various diets used by different workers showed a great variation in their composition. Since it seemed desirable to have as few outside factors as possible influencing the diet used, the following formula was devised:

	Per cent
Ground whole corn	70.0
Casein	11.0
Sucrose	15.0
Lard	2.0
Calcium carbonate	1.4
Sodium chloride	0.6
Total	100.0

The casein was prepared by taking a commercial casein and dissolving it in a 0.085 normal sodium hydroxide solution after it had been washed thoroughly with distilled water. The casein was then precipitated with an acid solution of a 1 to 1 mixture of normal hydrochloric and acetic acids. The precipitated casein was washed with distilled water until no test for chlorides could be observed; then it was dried in a

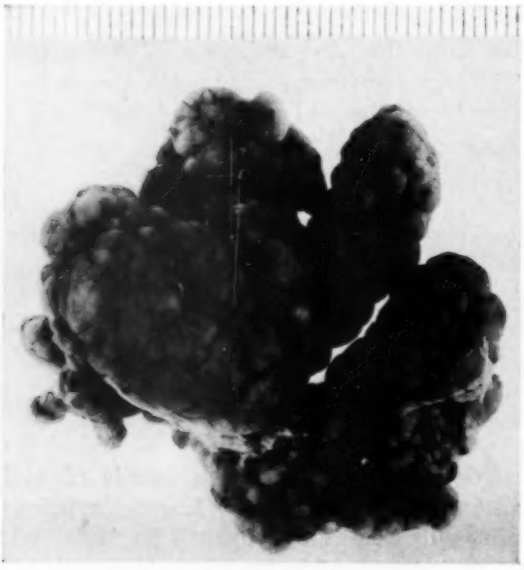

Fig. 4 Liver from rat no. 623F, which was killed after 340 days on a diet containing lethal wheat no. 459 plus 0.4 gm. yeast, 0.15 gm. cod liver oil, and 5 cc. of orange juice daily (series 20).

steam heated drier and ground. The sucrose used was a commercial cane sugar. The lard was Swift's Silver Leaf brand. The salts were chemically pure laboratory reagents. The control and experimental (contained corn lab. no. 388) diets varied only in the source of the corn.

Each rat was kept in an individual cage, as described by Burr and Burr ('29). The rats were weighed every 5 days and the daily food intake recorded.

The rats constituting series 1 were divided into four groups as evenly as possible in regard to age, litter and sex. The four groups in this series were fed as follows:

Group I Experimental corn no. 388 diet + distilled water.
Group II Control corn diet + distilled water.
Group III Experimental corn no. 388 diet + well water (local).
Group IV Control corn diet + well water (local).

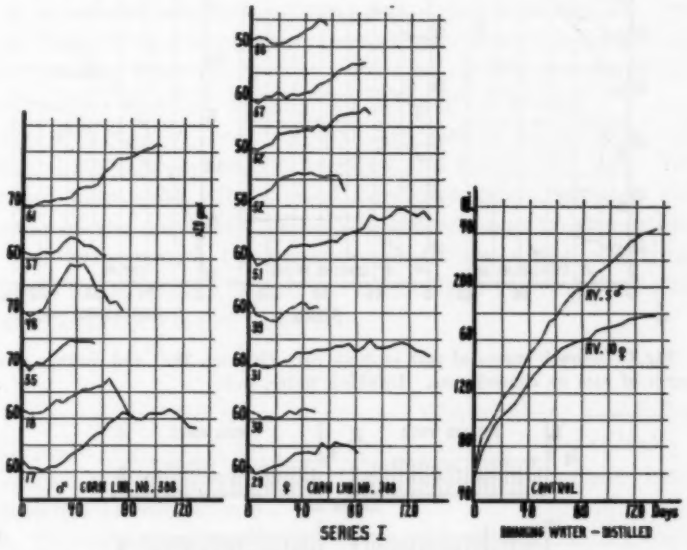

Fig. B Growth curves of rats on toxic corn (lab. no. 388) and average growth curve of rats on control corn. Drinking water, distilled.

The results obtained in series 1 are shown in figures B, C, and D. An examination of the charts indicates clearly that in every case the diet containing corn lab. no. 388 resulted not only in decreased growth, but also in death as well. The control animals showed good growth and were in fine physical condition at the end of the experiment. Figure C, showing the growth curves of the rats receiving well water, seems to show quite distinctly that these rats were more susceptible to the toxicant than were the rats on distilled water (fig. B).

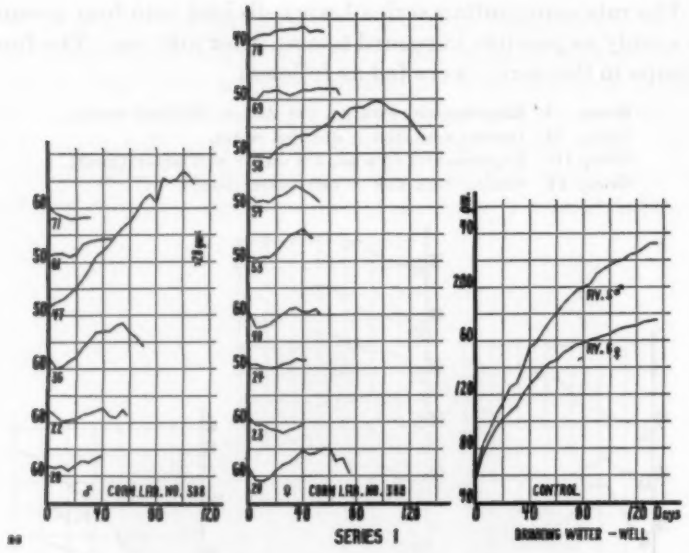

Fig. C Growth curves of rats on toxic corn (lab. no. 388) and average growth curve of rats on control corn. Drinking water, well.

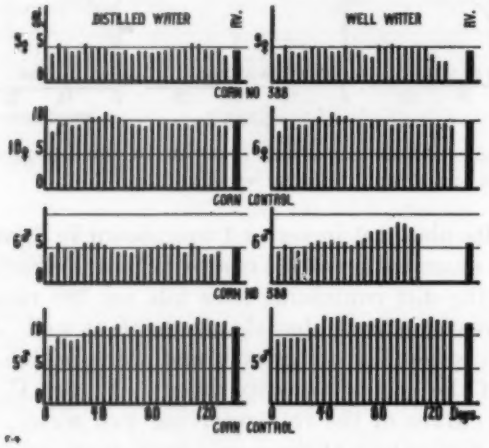

Fig. D The average food consumption of rats in 5-day periods per rat day, and average for entire period (series 1).

The average length of life of males and females receiving toxic food plus distilled water was 85.0 and 84.4 days, respectively, while those receiving toxic food plus well water lived 59.3 and 63.3 days respectively. Autopsies seemed to indicate that the lesions in the rats receiving well water were more severe. Further studies on this point will be made in the future.

As a record of food consumption (shown in fig. D) was kept daily, it was recognized immediately that the rats which obtained the diets containing the corn no. 388 consumed approximately one-half as much as the control groups.

The first rat on the toxic corn succumbed on the thirtieth day and the last on the one hundred and thirty-fifth day. The control rats were all in good condition when killed. The deaths of the experimental rats were not due to starvation, as has been shown by Franke and Potter ('34). Various degrees of pathology, which were described above were noted in the post-mortems of these rats.

SUMMARY

1. An apparently new nutritional disturbance, associated with plant material from particular localities, is described.

2. The effects are probably produced by a definite toxic compound or compounds.

3. Thirteen samples of various cereal grains, including corn, wheat, barley, and emmer, from the affected areas produced death in 299 out of 325 white rats within 100 days.

4. Definite pathologic effects produced by the toxic materials are described.

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A NEW TOXICANT OCCURRING NATURALLY IN CERTAIN SAMPLES OF PLANT FOODSTUFFS [1, 2]

II. THE OCCURRENCE OF THE TOXICANT IN THE PROTEIN FRACTION

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(Received for publication March 22, 1934)

FOUR FIGURES

After a large number of feeding trials had shown that certain grains produced a nutritional disturbance which could only be caused by some extremely toxic compound or compounds occurring naturally in the plants (Franke, '34) work was begun on the chemical isolation of the toxic factor.

In the summer of 1930, a preliminary study was begun to determine which fraction of the corn (no. 388) carried the toxicant. Corn was extracted with petroleum ether, distilled water, 5 per cent K_2SO_4 solution and 70 per cent ethyl alcohol. After feeding trials using residues from these extractions, it was felt that the protein fraction carried the toxicant. Since it is much easier to separate the protein of wheat, the next experiment was carried out with wheat sample no. 459 which feeding trials had shown to be lethal. This experiment (series 36) gave such conclusive results that it will be described here.

[1] Published with the permission of the director of the South Dakota Agricultural Experiment Station as communication no. 2 from the Department of Experiment Station Chemisty.

[2] These investigations are being carried out under the Purnell Fund and with the cooperation of the Bureau of Chemistry and Soils, Bureau of Plant Industry, Bureau of Animal Industry, and Bureau of Home Economics of the United States Department of Agriculture.

EXPERIMENTAL

Whole wheat was ground and the gluten separated from the bran and starch by repeatedly washing in small portions of distilled water. The starch and bran washings were combined and enough sodium hydroxide added to make 0.2 per cent sodium hydroxide solution. The mixture was stirred mechanically for about 4 hours. The suspension was centrifuged in a Sharples supercentrifuge, and the bran and starch were washed twice with distilled water, and dried. The 0.2 per cent sodium hydroxide solution containing the protein dissolved from the starch and bran was used to disperse the gluten. The resulting solution was centrifuged to remove the starch and bran particles. After thorough washing, these were added to the main body of starch and bran previously separated. The protein solution was precipitated at its iso-electric point by adding dilute hydrochloric acid, washed with distilled water twice, and dried.

Both control wheat and wheat no. 459 were treated in this manner and made up into diets (diet no. 3) having the following composition:

	Per cent
Whole ground wheat (separated bran and starch plus precipitated protein)	82.0
Commercial casein	10.0
McCollum's salt mixture no. 185	4.0
Cod liver oil	2.0
Northwestern dehydrated yeast	2.0
Total	100.0

But with the mixtures of protein, starch and bran as follows:

Group A No. 459 protein plus control starch and bran
Group B Control protein plus no. 459 starch and bran
Group C Control protein recombined with control starch and bran

The rats were kept in individual cages described by Franke and Franke ('34). The food intake was recorded daily and the body weight taken every 5 days.

Figures A and B show the individual growth curves of the rats receiving the protein from toxic wheat (group A), as

compared with the composite growth curves of the rats receiving bran and starch from toxic wheat (group B), and the rats receiving both control protein and control bran and starch (Group C). It is quite evident that the bulk of the toxicant is carried in the protein fraction. Experimental indi-

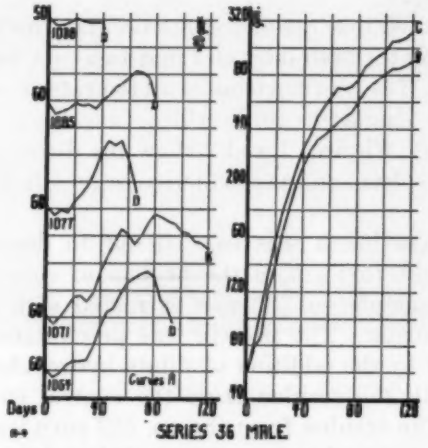

Fig. A Growth of males in series 36: Individual curves—Group A no. 459 protein plus control starch and bran. Average curve—Group B, control protein plus no. 459 starch and bran. Average curve—Group C, control protein recombined with control starch and bran.

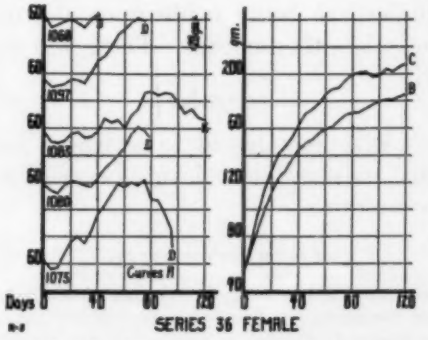

Fig. B Growth of females in series 36: Individual curves—Group A, no. 459 protein plus control starch and bran. Average curve—Group B, control protein plus no. 459 starch and bran. Average curve—Group C, control protein recombined with control starch and bran.

cations are that the growth depression shown in the case of the rats in group B is due to protein which remained in the bran and starch residue. Although the rats in this group showed no pathology, their growth curves are interesting in that they show the growth-depressing effect of small amounts of the toxicant.

It will be noted that the first death occurred on the 41st day, and the last on the 96th day, and that two rats were killed on the 120th day. The postmortems of all the rats showed pathological changes similar to those which have been noted before (Franke, '34). Figures 1 and 2 show the distinctive changes in the livers, spleen, and reproductive organs in typical males and females.

Another experiment was carried out to demonstrate the presence of the toxicant in the protein of corn (series 44). Lethal corn sample no. 523 was extracted with 0.2 per cent sodium hydroxide. The protein was precipitated at its isoelectric point by the addition of dilute hydrochloric acid and combined with the residue from the control corn similarly extracted. The residue from the no. 523 corn was then combined with the control corn protein and the mixture made into diets as in series 36 described above. The results here were not as clear cut as in the wheat experiment, but most of the toxicant was removed by the extraction.

Further studies are being made upon the toxic proteins in an effort to isolate the toxicant. These will be reported in a later paper.

SUMMARY

Experiments with samples of toxic wheat and toxic corn have shown that most of the toxicant is carried in the protein fraction of these grains.

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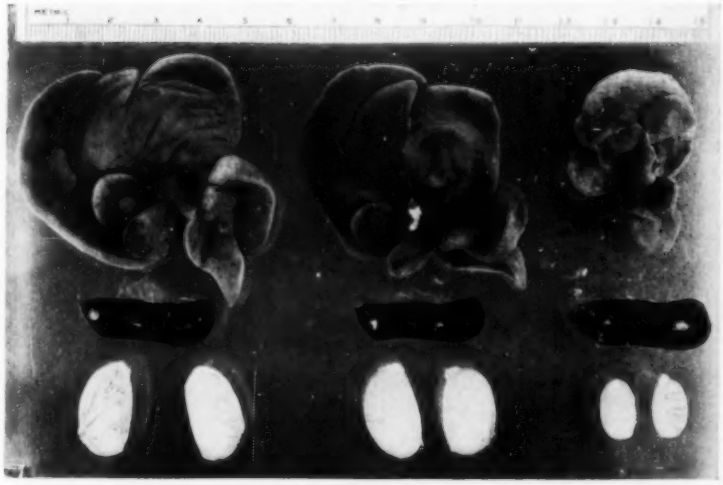

Fig. 1 Relative condition of the liver, spleen and testes from representative litter mates in series 36. Left to right: Group C, B, and A.

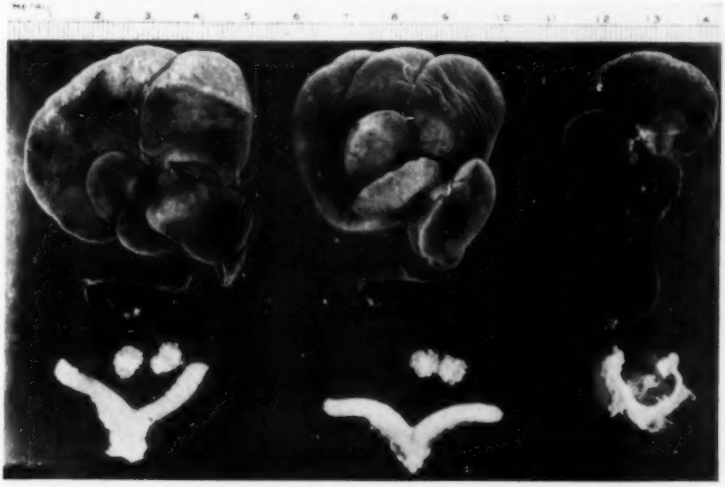

Fig. 2 Relative condition of the liver, spleen, uterus and ovaries from representative litter mates in series 36. Left to right: Groups C, B, and A.